DATE

The Honorable E. Scott Pruitt
Administrator
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, N.W.
Washington, DC 20460

Subject: Review of EPA's Draft Assessment entitled *Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (September 2016)*

Dear Administrator Pruitt:

The EPA's National Center for Environmental Assessment (NCEA) requested that the Science Advisory Board (SAB) review the draft assessment, entitled *Draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX).* The draft assessment consists of a review of available scientific literature on the toxicity of RDX. The SAB was asked to comment on the scientific soundness of the hazard and dose-response assessment of RDX-induced cancer and noncancer health effects. In response to EPA's request, the SAB convened a panel consisting of members of the SAB Chemical Assessment Advisory Committee (CAAC) augmented with subject matter experts to conduct the review.

The SAB finds the draft assessment to be comprehensive and generally well-written. The enclosed report provides the SAB's consensus advice and recommendations. This letter briefly conveys the major findings.

The draft assessment evaluates and modifies available physiological based pharmacokinetic (PBPK) models in the literature. The SAB finds the revised rat and human PBPK models to be a distinct improvement, and their use in the assessment for the calculation of human equivalent doses (HEDs) for the points of departure (PODs) for neurotoxicity and other noncancer endpoints to be scientifically supported.

For the hazard identification and dose-response assessment of noncancer endpoints, the SAB agrees that neurotoxicity, including seizures or convulsions, is a human hazard of RDX exposure. However, convulsions in rodents can only provide a limited spectrum of potential human hazard since convulsive or nonconvulsive seizures, epileptiform discharges, reduction in seizure threshold, subchronic sensitization, and neuronal damages can all be part of the spectrum of RDX's nervous system hazards. Thus, further evaluation or explanation should be provided in the draft assessment for these potential endpoints. The SAB agrees RDX-induced convulsions arise primarily through a mode of action resulting from RDX-induced $GABA_A$ receptor ($GABA_AR$) blockade. The SAB also agrees with the characterization of convulsions as a severe endpoint, and its potential relationship to mortality, is clearly described. However, the SAB does not agree with EPA's use of a benchmark response (BMR) of 1% for deriving the lower bound on the benchmark dose (BMDL) as the point of departure (POD) from Crouse et al. (2006). A BMR of 1% would correspond to a response that is a factor of 15 below the lowest observed response data. Instead, the SAB considers the use of BMR of 5% based on the Crouse study to be more consistent with the observed response at the Lowest-Observed-Adverse-Effect-Level (LOAEL) of 15%, and not so far below the observable data. In addition, while the 4% response at the LOAEL in Cholakis et al. (1980) is lower, it is still closer to a BMR of 5% than the BMR of 1% proposed in the draft assessment.

With respect to the application of uncertainty factors to the PODs, the SAB supports the application of an interspecies uncertainty factor of 3 to account for the toxicodynamic and residual toxicokinetic uncertainty in extrapolation from animal to human not accounted for by the toxicokinetic modeling. In addition, the SAB also agrees with the subchronic to chronic uncertainty factor of 1, the LOAEL to No-Observed-Adverse-Effect-Level (NOAEL) uncertainty factor of 1, and the uncertainty factor of 10 to account for intra-human variability. However, the SAB disagrees with the application of a database uncertainty factor (UF_D) of 3, and recommends EPA consider applying a UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe effects, and the proximity of BMDL01s for convulsions to LD01s for lethality. The composite uncertainty factor should be 300 instead of 100 proposed in the draft assessment.

The SAB concludes the derived reference dose (RfD) for nervous system effects is not scientifically supported as it did not capture all of the potential adverse nervous system outcomes or their severity. The SAB recommends the assessment use the NOAEL from the Cholakis study as the primary basis for the derivation of a RfD for neurotoxicity in addition to the dose-response data of the Crouse study.

The SAB agrees that kidney and other urogenital system toxicity are a potential human hazard of RDX exposure. However, the SAB disagrees with the selection of suppurative prostatitis as the “surrogate marker” to represent this hazard, and recommends that EPA considers suppurative prostatitis as a separate effect. As such, separate organ/system-specific RfDs should be derived for renal papillary necrosis and the associated renal inflammation and suppurative prostatitis.

The SAB disagrees with the conclusion that male reproductive effects are a human hazard associated with RDX exposure. The SAB concludes that RDX does not pose a teratogenic risk to humans based on animal data. Additionally, the SAB agrees that conclusions cannot be drawn regarding other forms of developmental toxicity, which were only seen at maternally toxic dose levels. The SAB also notes that potential neurodevelopmental toxicity based on the reported mechanism of RDX inhibition of GABAergic neurons and the findings that RDX is present in the brain of developing rats during gestation and lactation were not adequately discussed in the draft assessment. The SAB concludes the proposed RfD for reproductive system effects in the draft assessment is not scientifically supported.

With regard to cancer effects, the SAB agrees that “suggestive evidence of carcinogenic potential” is the most appropriate cancer hazard descriptor for RDX, in accordance with EPA’s *Guidelines for Carcinogen Risk Assessment*; and that this descriptor applies to all routes of exposure. The SAB also agrees with the agency’s rationale for a quantitative cancer dose-response analysis for RDX, and the use of the linear low-dose extrapolation approach since the mode of action for cancer is unknown. However, the SAB finds the calculations of the PODs and oral slope factor were not clearly described, and questions whether these are scientifically supported. The SAB recognizes the Agency’s preference for using the multistage model for cancer dose-response modeling. However, a number of concerns were expressed with the data used to derive the cancer POD, the rationale for restricting modeling to the multistage model to derive the POD, and the conditions under which the MS-COMBO methodology provides a valid POD and cancer slope factor estimate.

With regard to dose-response analysis, the SAB agrees the overall RfD should be based on nervous system effects. However, the SAB finds that the scientific support for the proposed overall RfD is weak. In particular, the choice of Crouse et al. (2006) (LOAEL of 8 mg/kg-day) as the study to derive the overall RfD does not address the confirmed convulsion in an exposed pregnant female at 2 mg/kg-day in Cholakis et al. (1980). Thus, the SAB recommends EPA use a NOAEL of 0.2 mg/kg-day from the Cholakis study as the POD to calculate the RfD.

For dose-response analysis of the proposed oral slope factor, the SAB has questions regarding the adequacy of scientific support for the derived oral slope factor, as noted above. The SAB makes multiple suggestions in this review on how the discussion on the derivation of the oral slope factor can be improved.

The SAB appreciates this opportunity to review EPA's *Draft Toxicological Review of RDX* and looks forward to the EPA's response to these recommendations.

Sincerely,

Dr. Peter S. Thorne
Chair
EPA Science Advisory Board

Dr. Kenneth S. Ramos
Chair
SAB Chemical Assessment Advisory Committee
Augmented for the Review of the Draft IRIS
RDX Assessment

Enclosure

NOTICE

U.S. Environmental Protection Agency
Science Advisory Board
Chemical Assessment Advisory Committee Augmented for the Review of Draft IRIS RDX Assessment

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[to be added]

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ABBREVIATIONS AND ACRONYMS

AIC	Akaike Information Criteria
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BDNF	brain-derived neurotrophic factor
BMC	benchmark concentration
BMCL	lower 95% confidence limit of the benchmark concentration
BMD	benchmark dose
BMDL	lower 95% confidence limit of the benchmark dose
BMR	benchmark response
BW	body weight
CAAC	Chemical Assessment Advisory Committee
CASRN	Chemical Abstracts Service Registry Number
CI	confidence interval
CID	chemical identification number
EPA	Environmental Protection Agency
GABA	gamma-amino butyric acid
$GABA_AR$	gamma-amino butyric acid type A receptor
HED	human equivalent dose
HERO	Health and Environmental Research Online
HMX	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
IARC	International Agency for Research on Cancer
ILSI	International Life Sciences Institute
IPSPs	inhibitory postsynaptic potentials
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
K_i	inhibition constant
LD	lethal dose
LD01	dose that causes death in 1% of exposed animals
LOAEL	Lowest-Observed-Adverse-Effect Level
miRNA	microRNA
MEDINA	methylenedinitramine
MNX	hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine
MOA	mode of action
NAS	National Academy of Sciences
NCI	National Cancer Institute
NIOSH	National Institute for Occupational Safety and Health
NOAEL	No-Observed-Adverse-Effect Level
NRC	National Research Council
NTP	National Toxicology Program
OR	odds ratio
ORD	Office of Research and Development
OSF	oral slope factor
PBPK	physiologically based pharmacokinetic

PND	postnatal day
POD	point of departure
PTX	picrotoxin
PWG	Pathology Working Group
RfC	reference concentration
ROS	reactive oxygen species
RR	relative risk
SAB	Science Advisory Board
SDMS	spontaneous death or moribund sacrifice
TNX	hexahydro-1,3,5-trinitroso-1,3,5-triazine
UCL	Upper Confidence Limit
UF	uncertainty factor
UF_D	Database uncertainty factor
UF_H	Human inter-individual variability uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WHO	World Health Organization

1. EXECUTIVE SUMMARY

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency's *Draft IRIS Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (September 2016)* (hereafter referred to as the draft assessment). EPA's IRIS is a program that evaluates information on human health effects that may result from exposure to environmental contaminants. The draft assessment consists of a review of the available scientific literature on RDX. The draft assessment was revised in September 2016 and a summary of EPA's disposition of the public comments received on an earlier draft version of the assessment was added in Appendix E of the Supplemental Information to the Toxicological Review.

Literature Search Strategy/Study Selection and Evaluation
In general, the literature search strategy, study selection considerations, and study evaluation considerations, including inclusion and exclusion criteria, are mostly well-described, documented, and appropriate. However, the SAB has identified several areas that EPA's literature search did not cover, including literature for the role of GABAergic systems in brain development and the potential developmental neurotoxicity of RDX which interferes with GABAergic systems. In addition, EPA should clarify in the literature search strategy section what was done with nonmammalian species studies and secondary references. The SAB also recommends EPA include a brief description of what is known about the mammalian toxicity of the RDX degradation products, including hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), The SAB has identified additional peer-reviewed studies from the literature, which the agency should consider in the draft assessment.

Toxicokinetic Modeling

The SAB finds the conclusions reached by the EPA following its evaluation of the PBPK models of Krishnan et al. (2009) and Sweeney et al. (2012a, b) to be well-documented and scientifically supported. The modifications to the PBPK models of Krishnan/Sweeney represent distinct improvements. The EPA also performed validation of the PBPK model using independent rat data sets, and provided goodness of fit parameters. The SAB finds the uncertainties in the model well described.

The SAB concludes that the choice of dose metric for neurotoxicity is clearly described. Without brain concentration data, plasma or blood concentration data is used as a surrogate for brain concentrations. This approach can be justified, since limited pharmacokinetic data in mice, rats, and swine and in a human show concordance between blood and brain RDX levels over time following exposure. The use of area under the curve (AUC) in a plasma concentration-time plot as a dose metric for interspecies extrapolation to humans from oral points of departure (PODs) derived from rat data is supported. AUC is representative of the average RDX plasma concentration over a dosing interval, i.e. 24-hour interval. Published 24-hour time-courses of blood and brain RDX levels in rats appear to coincide with symptomatology. The mouse model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. This decision is scientifically supported and clearly explained.

Hazard Identification and Dose-Response Assessment

Nervous System Effects

The available human, animal, and mechanistic studies support EPA's conclusions that neurotoxicity, including seizures or convulsions, are human hazards of RDX exposure. Furthermore, RDX-induced convulsions arise primarily through a rapid mode of action resulting from RDX-induced $GABA_A$ receptor ($GABA_AR$) blockade. Despite the limitations of the only cross-sectional study of Ma and Li (1993), which indicated significant neurobehavioral and memory deficits associated with RDX exposure for workers in a Chinese RDX plant, the sum of evidence from clinical case reports, results from experimental animals, and mechanistic studies of RDX, there is sufficient evidence to support this conclusion. Therefore, RDX should be considered for classification as a *potential convulsant or proconvulsant to humans.* However, the evidence presented in the draft assessment does not adequately depict RDX's hazards to the nervous system, that convulsions in rodents can only provide a limited spectrum of potential human hazard, and that convulsive or nonconvulsive seizures, epileptiform discharges, reduction in seizure threshold, subchronic sensitization, and neuronal damage can all be part of the spectrum of RDX's nervous system hazards. Additional studies addressing cognitive and behavioral effects of RDX would assist in assessing other endpoints less severe than convulsions. Although there is data from existing animal studies showing changes in behavior, the data are not sufficiently robust to evaluate dose-response relationships, and animal data on cognitive changes is lacking. Therefore, there are no endpoints in existing studies to address the complete spectrum of effects.

The SAB finds the selection of studies reporting nervous system effects scientifically supported and clearly described. It is appropriate to consider the dose-response data reported in Crouse et al. (2006) as a relevant model that should be predictive of the bounds of dietary exposure. While this study utilized gavage administration of RDX rather than a dietary route of administration, there is less variability in the amount of the toxic agent delivered than for dietary intake that is dependent on the animal's feeding habits. As long as these characteristics of administration are understood and accounted for, there is no reason to exclude the work using gavage routes of exposure. The SAB agrees the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, is appropriately described.

The SAB finds that the selection of convulsions as the endpoint to represent nervous system hazard for RDX is clearly described. Convulsion is the most sensitive biologically significant endpoint that has been reasonably and reliably measured for RDX. However, evidence from other seizurogenic compounds with similar mode of action suggests that there are other, generally sub-clinical cognitive and behavioral neurological effects that occur at doses that are 2-3 times lower than doses causing seizure. The SAB agrees that the likely dose range between convulsion and other nervous system effects can be addressed using uncertainty factor adjustments. The SAB also finds that the calculation of the HEDs using PBPK modeling for the convulsion studies in rats to be scientifically supported and clearly described, and endorses the approach of estimating the effective concentration as the area under the curve (AUC) of concentration and time.

However, the SAB does not agree with EPA's use of a BMR of 1% for benchmark dose modeling of the Crouse et al. (2006) data for convulsions. EPA's choice of a BMR of 1% for modeling is based on the severity of the convulsion endpoint and on the proximity (dose-wise) of convulsions to lethality. In the Crouse study, a BMR of 1% would correspond to a response that is a factor of 15 below the lowest observed response data. The SAB agrees that the proximity of these two endpoints is indeed a valid source of uncertainty in terms of providing sufficient protection for sensitive human populations. However, the SAB concludes that uncertainty about the appropriateness of the dose-response data and the POD derived from those data should be addressed through uncertainty factors and not through unsupported extrapolation of the dose-response data. A BMR of 5% based on the Crouse study is more consistent with the observed response at the Lowest-Observed-Adverse-Effect-Level (LOAEL) of 15% and not so far below the observable data. In addition, while the response at the LOAEL in the Cholakis (1980) study is lower at 2 mg/kg/day, it is still closer to a BMR of 5% than the BMR of 1% proposed in the draft assessment.

With respect to the application of uncertainty factors to the PODs, the SAB supports the application of an interspecies uncertainty factor of 3 to account for the toxicodynamic and residual toxicokinetic uncertainty in extrapolation from animal to human not accounted for by the toxicokinetic modeling, a subchronic to chronic uncertainty factor of 1, a LOAEL to No-Observed-Adverse-Effect (NOAEL) uncertainty factor of 1, and a factor of 10 for intra-human variability. However, the SAB disagrees with the application of a database uncertainty factor (UF_D) of 3, and recommends EPA consider applying a UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe effects, and the proximity of BMDL01s for convulsions to LD01s for lethality. The composite uncertainty factor should be 300 instead of 100 as proposed in the draft assessment.

The SAB does not find the reference dose (RfD) derived by EPA for nervous system effects to be scientifically supported and clearly the candidate RfD did not capture all of the potential adverse outcomes or their severity. The SAB recommends the draft assessment use the NOAEL from the Cholakis study as the primary basis for the derivation of the RfD for neurotoxicity, in addition to the dose-response data of the Crouse study.

Kidney and other Urogenital System Effects

The SAB agrees the available human, animal, and mechanistic studies support the conclusion that kidney and other urogenital system toxicity are a potential human hazard of RDX exposure. However, this conclusion is primarily supported by animal data, whereas available human studies that indicate the kidney as a potential target of RDX are sparse and only identify transient renal effects following acute human exposure. There are no reports of prostatic effects of RDX in humans and no pertinent mechanistic data regarding RDX effects on the kidney and urogenital system. The SAB finds all hazards to the kidney and urogenital system adequately assessed and described in the draft assessment, with the exception of the description of inflammatory changes in the rat prostate. The SAB also finds the selection of suppurative prostatitis as the endpoint to represent this hazard is clearly described in the draft assessment, but not scientifically supported because there is no known mechanistic link between suppurative prostatitis and renal papillary necrosis or adverse effects in the kidney.

The SAB finds the selection of the Levine et al. (1983) study on kidney and other urogenital system effects was clearly described, but not entirely supported by scientific evidence. Mild toxic effects of RDX exposure on the kidney were found in some species, but not others. In some studies, toxic effects were found in both sexes, while in others only male or female effects were observed. Of note is that some of these effects (i.e. mineralization) occurred in a small study with non-human primates, while some rodent studies did not find evidence of renal toxicity. Only in the chronic study of Levine et al. (1983) were severe toxic effects on the kidney found, and this was only seen in males at the highest dose (40 mg.kg-day); bladder toxicity also occurred in this treatment group, whereas effects on the prostate occurred at doses of 1.5 mg/kg-day and above. Therefore, the SAB determines that the selection of suppurative inflammation of the prostate as a "surrogate marker" of the observed renal and urogenital system effects for derivation of a reference dose to be not justified. The SAB recommends that a separate RfD be derived for renal papillary necrosis and the associated renal inflammation for the kidney and urogenital system and that the male accessory sex glands be designated as a separate organ system, with a separate RfD derived for suppurative prostatitis.

As for calculation of the POD and HED for suppurative prostatitis as a standalone endpoint, both are scientifically supported and clearly described. The application of uncertainty factors should be the same as those for nervous system effects, if this system-specific RfD is to be considered for selection as an overall RfD.

Developmental and Reproductive System Effects

The SAB disagrees with the conclusion in the draft assessment that there is suggestive evidence of male reproductive effects associated with RDX exposure. The available animal evidence based on testicular degeneration in male mice exposed to RDX in their diet for 24 months (Lish et al. 1984), is weak and does not support this conclusion. There is no human evidence indicating male reproductive toxicity; no human studies have focused on this question, and there were no incidental reports of reproductive effects following RDX exposures. The SAB also finds adequate evidence from animal studies to conclude that RDX does not pose a teratogenic risk to humans. In this regard, none of the developmental studies conducted in the rat and rabbit reported a teratogenic outcome. Additionally, the SAB agrees that conclusions cannot be drawn regarding other forms of developmental toxicity, which only occurred at maternally toxic dose levels. The SAB also concludes that RDX presents a potential neurodevelopmental hazard that was not adequately addressed in the draft assessment. A pilot developmental neurotoxicity study in rats found a significant concentration of RDX in the immature brain of offspring and in milk from dams treated with 6 mg/kg-day of RDX during gestation. Given that Lish et al. (1984) was used for the calculation of a POD and HED for the derivation of an organ/system-specific reference dose for reproductive system effects, the RfD based on testicular degeneration is not scientifically supported.

Other Noncancer Hazards

The SAB considers it important that the draft assessment be explicit as to whether the available evidence does or does not support liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects as a potential human hazard, and the rationale for reaching that conclusion. In addition, body weight gain should be included in this evaluation as it has been identified as a potential adverse effect of RDX exposure elsewhere.

Cancer

The SAB concurs with the EPA that "*suggestive evidence of carcinogenic potential*" is the most appropriate cancer hazard descriptor for RDX and that this descriptor applies to all routes of human exposure. The SAB agrees with the EPA that the relevant observations are the liver tumors that were observed in female B6C3F1 mice and male F344 rats and lung tumors that were observed in female B6C3F1 mice in two-year dietary bioassays (Lish et al. 1984; Levine et al. 1983). The SAB identified a number of limitations for these studies and determined that the evidence for a positive tumor response to RDX in two species, two sexes, or two sites, required by EPA's *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005) for a "*likely to be carcinogenic to humans"* descriptor, is weak or absent. On these bases, the SAB concludes that the descriptor, "*suggestive evidence of carcinogenic potential"*, is appropriate. The SAB also finds that the draft assessment adequately explains the rationale for a quantitative cancer dose-response analysis for RDX. Lish et al. (1984) was a well-conducted two-year bioassay that included a large number of animals tested at multiple dose levels, and increased incidences of neoplasms occurred in exposed female mice. The study is suitable and appropriate for dose-response assessment, consistent with EPA's 2005 *Guidelines for Carcinogen Risk Assessment*.

With regard to cancer dose-response assessment, the SAB supports the use of linear low-dose extrapolation approach as the mode of action for cancer for RDX is unknown. The SAB finds that the calculations of the PODs and oral slope factor are not clearly described, and the SAB has questions about whether these are scientifically supported. The SAB has concerns with the low incidence of liver tumors in female mice and its impact on dose-response modeling. The 1.5% incidence of liver tumors in the control B6C3F1 mice is unexpectedly low. In addition, the draft assessment relies on the Multistage model to describe the POD and cancer slope factor. While understanding the preference of the IRIS program for the multistage model form, the SAB recommends that at a minimum, the draft assessment discuss the adequacy of the fit of the multistage model to available data. The SAB also recommends that a more detailed description of the MS-COMBO methodology be provided in the draft assessment to include a description of the independence assumption and the impact of violations of this assumption on the estimated POD.

Dose-Response Analysis

Oral Reference Dose for Effects Other Than Cancer

The SAB finds the scientific support for the proposed overall RfD is weak. In particular, the choice of Crouse et al. (2006) as the basis for the overall RfD does not address the confirmed convulsion in an exposed pregnant female at 2 mg/kg-day reported in Cholakis et al. (1980). This LOAEL occurred at a dose below the LOAEL of 8 mg/kg-day in the Crouse study. The SAB recommends EPA use the NOAEL of 0.2 mg/kg-day from the Cholakis study as the POD to calculate the RfD. After converting the POD into HED using a PBPK derived adjustment factor of 0.487, and applying a composite uncertainty factor of 300 (as recommended by the SAB), the resulting RfD is 3×10^{-4} mg/kg-day.

Inhalation Reference Concentration for Effects other than Cancer

There are no toxicokinetic (TK) data from inhalation exposures of laboratory animals or humans to RDX. There are epidemiological studies of persons exposed occupationally to RDX, but no information was provided on exposure levels. In light of the lack of TK data and exposure levels, an inhalation reference concentration cannot be derived.

Oral Slope Factor for Cancer

The SAB finds that the calculation of an oral slope factor for cancer endpoints is not clearly described in the draft assessment, and has questions about whether the oral slope factor is scientifically supported. The SAB makes multiple suggestions on how the discussion can be improved.

Inhalation Unit Risk for Cancer

There are no toxicokinetic data from inhalation studies of RDX in laboratory animals or humans, no carcinogenicity bioassays of RDX, nor data on cancer incidence in humans. Therefore, an inhalation unit risk for cancer cannot be derived.

Executive Summary

Generally, the SAB considered the Executive Summary to be well written, succinct, and clear. As changes are made to the body of the draft assessment in response to the SAB's recommendations, the Executive Summary should be updated accordingly. In addition, the SAB offers a number of suggestions for improving the Executive Summary.

2. INTRODUCTION

The Science Advisory Board (SAB) was asked by the EPA Integrated Risk Information System (IRIS) program to review the agency's *Draft IRIS Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)* (hereafter referred to as the draft assessment). EPA's IRIS is a human health assessment program that evaluates information on health effects that may result from exposure to environmental contaminants. The draft assessment consists of a review of available scientific literature on RDX. The draft assessment was revised in September 2016 and a summary of EPA's disposition of the public comments received on an earlier version of the assessment was added in Appendix E of the Supplemental Information to the Toxicological Review.

In response to the EPA's request, the SAB convened an expert panel consisting of members of the Chemical Assessment Advisory Committee augmented with subject matter experts to conduct the review. The SAB panel held a teleconference on November 17, 2016, to discuss EPA's charge questions (see Appendix A), and a face-to-face meeting on December 12 - 14, 2016, to discuss responses to charge questions and consider public comments. The SAB panel also held teleconferences to discuss their draft reports on ---, and ---. Oral and written public comments have been considered throughout the advisory process.

This report is organized to follow the order of the charge questions. The full charge to the SAB is provided as Appendix A. Editorial comments from the SAB are provided in Appendix B. The SAB also provides suggestions on the format of EPA's charge questions in Appendix C.

3. RESPONSES TO EPA'S CHARGE QUESTIONS

3.1. Literature Search/Study Selection and Evaluation

Charge Question 1. The section on Literature Search Strategy| Study Selection and Evaluation describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

The literature search strategy, study selection considerations, and study evaluation considerations, including inclusion and exclusion criteria, are mostly well-described, documented, and appropriate, with a few exceptions noted below. EPA suitably cast a wide net to retrieve all pertinent studies for the evaluation of health effects associated with RDX exposure. They searched PubMed, Toxline, Toxcenter, Toxic Substances Control Act Test Submissions (TSCATS), and the Defense Technical Information Center (DTIC) database, a central online repository of defense-related scientific and technical information within the Department of Defense. Studies were then screened to find those relevant to assessing the adverse health effects of exposure and developing a dose-response assessment. Citations in review articles and citations within original articles were also obtained and screened for additional pertinent information.

Figure LS-1 and Table LS-1 provide a summary of the general inclusion and exclusion criteria for studies that were kept for further evaluation of potential health effects of RDX. EPA used criteria to exclude studies such as citations that were abstract only, on treatment and mitigation of environmental contamination with RDX, on laboratory methods, and those on the physical-chemical properties including explosivity. These were appropriate exclusion criteria, in the SAB's opinion. These criteria resulted in the exclusion of over 900 references from further evaluation. The SAB thought that Figure LS-1 could be clearer and better coordinated with the inclusion and exclusion criteria described in Table LS-1.

Table LS-1 indicates that studies on "ecological species" and nonmammalian species were also excluded. This seems to contradict statements (page xxix, lines 13-16) indicating that studies on nonmammalian species and ecosystem effects were considered as sources of information for the health effects assessment. The SAB suggests that these statements be clarified, and that data for all mammalian species be retained, even if they are considered "ecological species."
The SAB notes that the exclusion of nonmammalian species may not be appropriate in light of the use of nonmammalian species such as zebrafish (e.g., in medium throughput assays for developmental neurotoxicity) to evaluate potential health risk to humans, and describe Adverse Outcome Pathways. Although there may be no studies of RDX *in vitro* or in the cellular and tissue-based high throughput assays, future research using these types of assays may provide mechanistic information for chemicals that could be used in health effects assessments.
The SAB points out that the exclusion of data for reasons of RDX purity are not well supported, and that exposure to impurities occurs in real life settings. In some cases, water was the impurity, which is needed to minimize ignition hazard.

Inclusion criteria in Table LS-1 were related to whether a citation was a source of health effects data pertinent to assessing the risk to humans (e.g., studies of health outcomes in RDX exposed humans or standard mammalian models by either the oral or inhalation route; exposure to RDX measured; health outcomes/endpoints reported). Sources of mechanistic and toxicokinetic data were also included. Secondary references and other sources that described ecosystem effects, exposure levels, dealt with mixtures or were reviews or risk assessments and regulatory documents were excluded from study evaluation. However, EPA indicates that secondary references containing health effects data, and citations on nonmammalian toxicity were kept for consideration in the draft assessment. The description of what was done with secondary references could be clearer and coordinated between the text and Figure LS-1 and Table LS-2. EPA provides details of the search in Appendix B, including search terms, and the number of hits per search term sequence per database searched. They also tabulate the number of citations added to the database from their forward and backward web of science search of specific citations. Thus, the Agency has been transparent in its process of identifying studies for evaluation.

EPA's evaluation of studies is fairly well-described and summarized in Table LS-3. The Agency used standard criteria and questions to evaluate study quality and utility that are described in several EPA guidance documents cited in the draft assessment. Studies were evaluated considering the experimental design and conduct, issues around exposure to RDX, endpoints evaluated, and presentation of results. EPA describes generally the issues they considered in evaluating the utility of both human and animal studies to inform both hazard identification and dose-response assessment.

EPA excluded four studies on health effects and described the reason for excluding these in Table LS-2. Similarly, EPA describes some of the important limitations in experimental animal studies in Table LS-5. Overall, the description of EPA's study evaluation is clear, although the terminology is somewhat inconsistent (e.g., methodological features in Table LS-4 do not quite match the subheadings where these are described later in the section). Some details on strengths and limitations of specific studies chosen for further evaluation are provided in subsequent sections describing hazard identification and dose-response assessment for specific organ systems.

The SAB raised concerns about an inadequate description and discussion of supporting evidence for sensitive subpopulations in the draft assessment. Although there are no adequate studies on developmental neurotoxicity of RDX, there are some mechanistic studies implicating GABA antagonist activity of RDX in the neurotoxicity observed in animals and humans. The SAB concluded it would have been appropriate to search the literature for the role of GABA in brain development to describe what is known to date and incorporate this information into the draft assessment. Such mechanistic information provides evidence for the existence of sensitive subpopulations (e.g., infants, children, pregnant women and their fetus), and informs the choice of uncertainty factors meant to account for variability in the human population. EPA does not discuss the role of GABAergic systems in neurodevelopment and the potential for interference with this system by RDX (or other compounds with similar molecular mechanisms) to induce developmental neurotoxicity, an omission that should be rectified. The SAB identified six references that may be used to start the discussion of the role of GABAergic systems during development and the potential for RDX developmental neurotoxicity. A listing of these references is provided below.

The SAB also notes the lack of description of toxicity of some of the breakdown products described in the draft assessment. A brief description of toxicity of hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) (CID: 535289; CAS RN 5755-27-1) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) (CID: 26368; CAS RN 13980-04-6) should be included in the draft assessment. Although these are minor metabolites in aerobic systems, some reductive transformation products of RDX are present in ground waters near munitions and training facilities, and some are produced in the GI tract of mammals, present in the blood and target tissues of dosed mammals. As noted in the supplemental document, MNX and TNX are weakly mutagenic in Salmonella assays. In addition, the anaerobic bacteria metabolite, methylenedinitramine (MEDINA), the mammalian oxidative transformation product 4-nitro-2,4-diazabutanal (NDAB), and 4-nitro-2,4-diazabutanamide should be noted in the draft assessment. The SAB assembled 15 candidate references that address the toxicity of reductive transformation products, and studies that were conducted in species that may inform the current RDX assessment. A listing of these references is provided below.

Recommendations

- EPA should include a literature search on the role of GABAergic systems in brain development, and how this can inform a better understanding of the potential developmental neurotoxicity of RDX.
- EPA should include a brief description of what is known about the mammalian toxicity of the degradation products TNX, and MNX, which are present in experimental animals following RDX exposure.
- MEDINA and related oxidative transformation products should be discussed in the assessment.
- EPA should not automatically exclude nonmammalian species as they may bring important mechanistic insight into the draft assessment.
- EPA should clarify in the literature search strategy section what was done with nonmammalian species studies and secondary references.

Additional Citations for USEPA to Consider:

Coleman, NV, Spain JC, Duxbury, T. (2002). Evidence that RDX biodegradation by Rhodococcus strain DN22 is plasmid-borne and involves a cytochrome p-450. *J Appl Microbiol* 93: 463-472.

Creeley, CE. (2016) From drug-induced developmental neural apoptosis to pediatric anesthetic neurotoxicity – where are we now? *Brain Sci* 6(3):32-44.

Eaton, HL; DeLorme, M; Craig, AM. (2009). Metabolism of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Ovine Ruminal Microbes. *Microbial Ecol* 57: 569-569.

Fellows, RJ: Driver, CR; Cataldo DA, Harvey SD. (2006). Bioavailability of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the prairie vole (Microtus ochrogaster*). Environ Toxicol Chem* 25: 1881-1886.

Garcia-Reyero, N; Habib, T; Pirooznia, M; Gust KA, Gong, P; Warner, C; Wilbanks, M; Perkins, E. (2011). Conserved toxic responses across divergent phylogenetic lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics. *Ecotoxicology* 20: 580-594.

Gust, KA; Brasfield, SM; Stanley, JK; Wilbanks, MS; Chappell, P; Perkins, EJ; Lotufo, GR; Lance, RF. (2011). Genomic investigation of year-long and multigenerational exposures of fathead minnow to the munitiont compound RDX. *Environ Toxicol Chem* 30: 1852-1864.

Halasz, A, Manno D, Perreault NN, Sabbadin F, Bruce NC, Hawari J. (2012). Biodegradation of RDX Nitroso Products MNX and TNX by Cytochrome P450 XplA. *Environ Sci Technol* 46: 7245-7251.

Jaligama, S, Kale VM, Wilbanks MS, Perkins EJ, Meyer SA. (2013). Delayed myelosuppression with acute exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and environmental degradation product hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) in rats. *Toxicol Appl Pharmacol* 266: 443-451.

Jeilani YA, Duncan KA, Newallo DS, Thompson AN, Jr., Bose NK. (2015). Tandem mass spectrometry and density functional theory of RDX fragmentation pathways: Role of ion-molecule complexes in loss of NO3 and lack of molecular ion peak. *Rapid Commun Mass* Sp 29: 802-810.

Kim JY; Liu, CY; Zhang, F; Duan, X; Wen, Z; Song, J; Feighery, E; Lu, B; Rujescu ,D; St Clair, D; Christian, K; Callicot, JH; Weinberger, DR; Song, H; Ming, Gl. (2012). Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell* 148:1051-1064

Marty, S; Wehrle, R; Sotelo, C. (2000). Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. *The Journal of Neuroscience* 20:8087-8095.

Meyer, SA; Marchand AJ; Hight JL; Roberts, GH; Escalon, LB; Inouye, LS; MacMillan, DK. (2005). Up-and-down procedure (UDP) determinations of acute oral toxicity of nitroso degradation products of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *J Appl Toxicol* 25: 427-434.

Mukhi, S; Patino, R. (2008). Effects of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in zebrafish: General and reproductive toxicity. *Chemosphere* 72: 726-732.

Neal, AP; Guilarte, TR. (2013). Mechanism of lead and manganese neurotoxicity. *Toxicol Res* 2(2):99-114.

Pan, X; Ochoa, KM; San Francisco; MJ; Cox, SB, Dixon, K, Anderson, TA, Cobb, GP. (2013). Absorption, distribution, and biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine in B6C3F1 mice (Mus musculus*). Environ Toxicol Chem* 32: 1295-1303.

Rivera, C; Voipio J; Payne JA; Ruusuvuori, E; Lahtinen, H; Lamsa, K; Pirvola, U; Saarma, M; Kaila, K. (1999). The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397(6716):251-5.

Schneider, NR; Bradley, SL; Andersen, ME. (1978). Distribution and metabolism of cyclotrimethylenetrinitramine (RDX) in rat after sub-chronic administration. *Toxicol Appl Pharmacolog*y 46: 163-171.

Smith, JN; Pan, XP; Gentles, A; Smith, EE; Cox, SB; Cobb, GE. (2006). Reproductive effects of hexahydro-1,3,5-trinitroso-1,3,5-triazine in deer mice (Peromyscus maniculatus) during a controlled exposure study. *Environ Toxicol Chem* 25: 446-451.

Smith, JN; Espino, MA; Liu, J; Romero, NA, Cox, SB, Cobb, GP. (2009). Multigenerational effects in deer mice (Peromyscus maniculatus) exposed to hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). *Chemosphere* 75: 910-914.

Williams, LR; Wong, K; Stewart, A; Suciu, C; Gaikwad, S; Wu, N; DiLeo, J; Grossman, L; Cachat, J; Har,t P; Kalueff, AV. (2012). Behavioral and physiological effects of RDX on adult zebrafish. *Comparative Biochemistry and Physiology C-Toxicology and Pharmacology* 155:33-38.

Wirbisky, SE; Weber, GJ; Lee, JW; Cannon, JR; Freeman, JL. (2014) Novel dose-dependent alterations in excitatory GABA during embryonic development associated with lead (Pb) neurotoxicity. *Toxicology Letters* 229:1-8.

3.2. <u>Toxicokinetic Modeling</u>

In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al., 2012a; Sweeney et al., 2012b).

3.2.1. Model Evaluation

Charge Question 2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

The conclusions reached by the EPA following its evaluation of the PBPK models of Krishnan et al. (2009) and Sweeney et al. (2012a, b) are well-documented and scientifically supported. EPA did a thorough and accurate job reviewing and summarizing much of what is known about the oral absorption of different forms/preparations of RDX, as well as the compound's distribution, metabolism and excretion. The changes made to the PBPK model of Krishnan /Sweeney represent distinct improvements over the original approach. Human metabolic rate constants were fitted from human data. Additionally, it is stated that *in vitro* data from rats and human metabolic studies were used and scale-up to liver size based on microsomal protein. The EPA also performed validation of the PBPK model using independent rat data sets, and provided goodness-of-fit parameters. The uncertainties in the model are well-described. Overall, the draft assessment does an excellent job in compiling the data presented in Appendix C.

The SAB observed the following in its review of this section:

- Distribution Section C.1.2 should include the tissue parent and metabolite data of Pan et al. (2013) cited elsewhere in the report.
- More attention could be devoted in Section C.1.2. to describing the distribution of RDX to the brain as a key target tissue. Brain extracellular fluid concentration-effect relationships would be most informative. Changes in plasma/blood concentrations over time may be linearly related (proportional) to brain concentrations, and may be used to derive toxicity, as proposed, based on limited correlations observed with brain and plasma data from animal studies and data from a child after poisoning (Woody et al., 1986). The EPA chose not to use PBPK-simulated brain RDX concentrations, which were only moderately well fitted in Figure C-6, as a dosimeter for neurotoxicity risk assessment. Experimental findings lend support to the decision to use plasma as a surrogate.
- Protein binding of RDX is not mentioned in the draft assessment. This may be regarded as a potential weakness given that it is the free concentration that would diffuse across the blood-brain barrier in the absence of any active uptake processes or be available for metabolism. This could lead to differences in predicted brain/blood ratios in humans, and may be helpful in allometric scale-up of clearance. However, absent any empirical values for protein binding, use of total, rather than free, concentrations is the only option.
- Despite improvements in the model, the rat data are only moderately well fitted and show substantial deviations, especially at early time-points. This may reflect deviations of the simulations due to inaccurate model absorption parameters, and possibly imprecise clearance parameters. Further optimization may improve fitting. Insight into the nature of gastrointestinal absorption could be gained from *in vitro* studies using Caco2 cells or other intestinal models. For elimination, hepatic intrinsic clearance is preferred over a rate constant. From the *in vitro* microsomal and S9 studies reported by Cao et al. (2008), data are provided that can be used to calculate metabolic intrinsic clearance. The Cao study demonstrated that the intrinsic metabolic clearance in a microsomal preparation was greater in humans than in rats and mice. However, concentration-dependent studies were not performed, so this publication does not provide support for the assumption of linear clearance.
- Clearance terms instead of first order rate constants (dependent on elimination and the apparent volume of distribution) would be more informative in the model. *In vitro* (Km/Vmax or intrinsic metabolic clearance) or derivation of intrinsic clearance from fitted clearance obtained from *in vivo* data may be used.
- The role of metabolites in toxicity is discussed in the draft assessment, but due to a lack of data not included in the model. This is appropriate, though limited information on metabolites in brain and other tissues (Pan et al. 2013) indicates they could contribute to the

observed effects. The parent AUC dose metric would thus serve as an indicator of exposures to parent and metabolites, though not directly tracking the metabolites.

- Provision for tissue partitioning is mainly via *in silico* methods; more *in vivo* data would provide justification for these values.
- The mouse data are the least comprehensive, though EPA could re-evaluate whether the Guo et al. (1985) total radioactivity data are consistent with the Sweeney et al. (2012) data. The description of the noncompartmental analysis (page C-28) is good to provide perspective on the data versus the PBPK modeling.

Recommendations

While different modelers have in the past and might in the future make somewhat different choices to address the available pharmacokinetic data, the current modeling as performed is reasonable. No changes are recommended except to revise the text to address some of the above bullets, such as increased text on brain distribution.

3.2.2. Selection of Dose Metric

Charge Question 2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

For neurotoxicity, the choice of dose metric is clearly described (pages 2-8 and 2-9). The choice is reasonable given less than ideal data on the pharmacokinetic-pharmacodynamic (PK/PD) relationship for this endpoint. A PK/PD model likely would be driven by the concentration in brain that is responsible for the PD (neurotoxicity); brain RDX concentrations are derived from the blood-brain partitioning of RDX blood concentrations. Without brain concentration data, plasma or blood is used as a surrogate for brain concentrations. This can be justified, since limited PK data in mice, rats, and swine (Table C-1) and in a human (Woody et al. 1986) show concordance between blood and brain RDX levels over time following exposure, supporting the use of blood/plasma concentrations as a surrogate for brain concentrations, and for the use of plasma concentration-time curve AUC values as a dose metric.

AUC is representative of the average RDX plasma concentration over a dosing interval, i.e., 24-hour interval. Published 24-hour time courses of blood and brain RDX levels in rats (e.g., Bannon et al., 2009) appear to coincide with symptomatology, providing support for the use of AUC. It may be reasonable to assume that seizures or hyperreactivity would be manifest as long as a threshold blood/brain concentration of RDX, e.g., 8 μg/g (Williams et al. 2011) is present. Therefore, there is clear rationale for choosing AUC over peak plasma concentrations (C_{max}) values as the dose metric.

Since the POD_{HED} is presented in Table 2-2 in the draft assessment for both dose metrics for neurotoxicity, it can be seen that the difference between C_{max} and AUC/24 hour values is relatively modest in the rat (~30%). It should also be pointed out in the text on page 2-8 that AUC appears to be a better representation of the adverse effect of interest than is RDX concentration at a single point in time. Additionally, it should be noted that maximal plasma concentrations are not predicted well from the PBPK model, producing uncertainty in C_{max} values, and supporting the case for the use of AUC.

There does not appear to be an explanation for the choice of dose metric for the prostatitis endpoint, though some comments (e.g., AUC considered better estimated than C_{max} from PBPK model) in the discussion for neurotoxicity apply across endpoints. Again the differences in Table 2-2 are modest, and since this is an effect only observed in a chronic study, average daily AUC is a reasonable choice.

It is noted that although there are mechanistic data supporting the role of RDX in neurotoxicity (convulsions) through binding to $GABA_AR$ (Williams et al. 2011; Williams and Bannon, 2009), the effect of RDX might be due to either parent compound or metabolites; as such, any PK parameter that measures parent compound plasma concentrations may not accurately predict toxicity.

The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. This decision is scientifically supported and clearly explained on pages C-30 and C-31. The mouse model is more uncertain as discussed on page 2-9 of the draft assessment. The uncertainties are numerous.

3.2.3. Intrahuman Variation

Charge Quesiton 2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

It is standard practice to adopt an intraspecies factor of 10 to account for potential differences in the toxicokinetics and toxicodynamics of a chemical in the absence of information about variability within human populations. There is a paucity of data on the toxicokinetics, toxicodynamics or toxicity of RDX in humans. Given these extreme data limitations, it would not be reasonable to use a PBPK model to assess human variability.

Sensitivity analyses (described in Appendix C) showed that the PBPK model output was substantially impacted by bioavailability and by metabolic clearance. There are apparently no data to define the absorption phase following RDX ingestion by humans or animals. toxicokinetic data for RDX elimination by humans are quite sparse. It appears from two studies (Bhushan et al. 2003; Major et al. 2007) that RDX metabolism in some mammals is mediated by cytochrome P450s (CYPs). As the activities of CYPs and other enzymes that metabolize xenobiotics vary significantly in the human population, the rate of metabolic clearance of RDX would also be expected to vary. Potential inter-subject differences in formation of RDX metabolites may also contribute to uncertainty, should specific metabolites be associated with toxicities.

In light of the role of binding of RDX to the $GABA_AR$ in neurotoxicity, data on inter-subject variability in receptor binding and response could identify and characterize sensitive subpopulations. Thus, likely toxicodynamics and toxicokinetics differences support a full UF_H of 10.

3.3. <u>Hazard Identification and Dose-Response Assessment</u>

3.3.1. Nervous System Effects

3.3.1.1. Nervous System Hazard

Charge Question 3a(i). The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

The SAB agrees that available human, animal, and mechanistic studies support the conclusion that nervous system toxicity is a human hazard of RDX exposure.

Human Studies
There is consistent evidence from more than 20 clinical case reports that exposure to RDX is associated with adverse neurological outcomes, particularly an association with convulsions. Nevertheless, there are many and varied limitations to deducing the hazards of RDX solely based on such case reports. There is only one cross-sectional study that provides a snapshot of the potential neurotoxicity associated with inhalation exposure to RDX. Ma and Li (1993) presented results from a neurobehavioral test battery that also assessed memory retention on a study of workers exposed in a Chinese RDX plant at a single point in time. The results indicated significant neurobehavioral and memory deficits were associated with RDX exposure measured in air. However, this study has several significant limitations that impact any conclusions about RDX hazard solely based on its findings. Of greatest concern were: 1) the omission of exposure levels in the "non-exposed" group; 2) lack of attempt to control for confounders (non-occupational exposures, lifestyle, co-morbidity), and; 3) lack of a rationale for subdividing the exposed cohort. Nevertheless, the outcomes on Composite Memory Retention Quotient and Composite Block Design score were greater >15 points and >2 seconds lower ($p<0.01$) than the control group, respectively. Statistical analyses seem appropriate, but 95% confidence intervals would have been helpful since the magnitude of functional impairments across groups is within the High average [110-119] and Average [90-109] range, measures typically associated with a 15% Standard Deviation. Other studies are generally supportive with the strongest evidence for convulsions coming from investigations involving acute exposures (Testud, 1996; Hollander, 1969; Merrill, 1968).

Animal Studies
Several studies with rodents using oral gavage and dietary exposure models over the acute (Burdette 1988), sub-chronic (Crouse 2006, Von Oettingen 1949) and chronic (Lish 1984, Levine 1983, Hart 1976) timeframes have consistently identified a broad range of neurological impairments ranging in severity from irritability to tremors and other signs that may be considered prodromal of convulsions. Convulsive (seizure) activity is a common finding in most,

but not all, studies. In addition to seizure activity, several of these studies (Levine et al.1990; Angerhofer et al. 1986; Levine et al.1983; Levine et al. 1981; von Oettingen et al. 1949) identified "less severe" neurological and behavioral impairments that may be consistent with the sparser literature on human exposures. An important observation stemming from some of the animal studies is that RDX appears to sensitize animals exposed to lower doses to subsequent seizurogenic stimuli, including electrogenic, audiogenic, and chemical kindling. Another potentially important experimental finding is that the dose appears to be a more important predictor of adverse neurologic outcomes than is the duration of treatment.

Mechanistic Studies

The neurotoxicity profile of RDX is consistent with that of a centrally acting excitotoxicant. There is ample evidence of a direct interaction of RDX with $GABA_AR$ in the mammalian central nervous system. RDX blocks GABA-activated chloride ion currents and the inhibitory postsynaptic potentials (IPSPs) that form critical inhibitory networks throughout the brain. The available data do not preclude the influence of other receptors as yet unstudied for RDX but also implicate the limbic system, including the amygdala, as especially sensitive targets of RDX. Surprisingly, the potency of RDX as a $GABA_AR$ blocker is relatively low. By comparison, picrotoxin (PTX) has >100-fold lower K_i (100x more potent) than RDX at binding to $GABA_AR$ K_i (inhibition constant) of 0.2 vs. 21 μM) (Williams et al., 2011). The lower potency of RDX extends to the concentrations needed to inhibit chloride ion currents in whole cell voltage clamp experiments and inhibitory postsynaptic current (IPSC) events, which typically require >10μM. Also relevant to the RDX mechanism and its potential importance to long-term behavioral toxicity is the observation that the inhibitory actions of RDX on seizure-like neuronal discharges can be measured in the basolateral nucleus of amygdala (Williams et al. 2012). In contrast, evidence supporting a role for glutamate in the effects of RDX is limited, and a basis for excessive glutamate stimulation in the draft assessment is weak, if not unfounded.

Preliminary Conclusions

With regard to nervous system hazard identification, the available human, animal, and mechanistic studies support EPA's conclusions that neurotoxicity, including seizures or convulsions, are human hazards of RDX exposure. Furthermore, RDX-induced convulsions arise primarily through a rapid mode of action resulting from RDX-induced $GABA_AR$ blockade. Despite the limitations of the Ma and Li (1993) study, the sum of evidence from clinical case reports, results from experimental animals, and mechanistic studies of RDX, there is sufficient evidence to support this conclusion. Therefore, RDX should be considered for classification as a *potential convulsant or proconvulsant to humans.*

The evidence presented in the draft assessment, however, does not fully depict RDX's hazards to the nervous system: convulsions in rodents only provide a limited spectrum of potential human hazard, with convulsive or nonconvulsive seizures, epileptiform discharges, reduction in seizure threshold, subchronic sensitization, and neuronal damage all being part of the spectrum of RDX's nervous system hazards. Further evaluation or explanation should be provided for these potential endpoints. With respect to whether all hazards to the nervous system were adequately assessed, the measure of abnormal electrographic activity or seizure-like activity in specific brain regions may be a more sensitive indicator of neurotoxicity than the potential of RDX to elicit subtler neurological impairments such as cognitive deficits and/or behavioral abnormalities.

Endpoints such as convulsions, tremors and aggression are appropriate as part of the spectrum of effects. Additional studies addressing cognitive and behavioral effects of RDX would assist in assessing other endpoints less severe than convulsions. Although there are data from existing animal studies showing changes in behavior, the data are not sufficiently robust to evaluate dose-response relationships, and animal data on cognitive changes are lacking. Given these limitations, additional endpoints are needed to address the complete spectrum of effects.

3.3.1.2. Nervous System-Specific Toxicity Values

Charge Question 3a(ii). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

The selection of studies reporting nervous system effects is scientifically supported, clearly described, and sufficient to draw conclusions about the potential hazards associated with exposures to RDX. By reviewing the scope of the search strategy and the process for identifying studies that report health effects and meet appropriate standards of quality for conduct, design, and reporting, it is concluded that the most reliable scientific information has been accessed for this draft assessment.

For assessment of convulsant effects, the neurotoxic endpoints, and in particular the convulsion endpoints, are appropriate for revealing the hazards of RDX delivered by oral gavage administration (Crouse et al. 2006). The differences in toxicokinetics of RDX exposure by gavage versus dietary administration are clear, and must be accounted for when predicting risk. This and several other studies in the select group reporting effects on neurological health utilized gavage administration as opposed to a dietary route of administration. The evidence indicates that the gavage route results in higher peak blood and brain levels of RDX than the dietary route and that the rate of rise in blood and brain levels is faster with gavage. This likely reflects a lesser degree of variability in the amount of the toxic agent absorbed compared to the dependence of dietary intake on animal's feeding habits. Since it is the dose rather than the duration of exposure that is more predictive of neurological outcomes, at least from animal studies, the SAB concludes that it is appropriate to consider the dose–response data reported in the Crouse study as a relevant model. In fact, the Crouse study produced perhaps the best RDX dose-response data available.

The SAB agrees that the characterization of convulsions as a severe endpoint, and its potential relationship to mortality, are appropriately described. Based on the available data, death may occur without seizure or convulsions, although this may simply be due to a low frequency of observations. However, based on the current state of science (including the epilepsy literature), death is not a necessary outcome of seizures or convulsions, and is driven by abnormal electrographic patterns in the brain. While the relationship between convulsions and mortality is unclear in the overall scheme of assessment of neurotoxicity endpoints for RDX, it is nonetheless reasonable to conclude that convulsions, as characterized in the draft assessment, represent a reasonable severe endpoint for human health risk assessment. In addition, more consideration should be given to available data on fatal outcomes and the possibility that mortality may arise from non-nervous system factors or hazards.

3.3.1.3. Points of Departure for Nervous System Endpoints.

Charge Question 3a(iii). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described? Is the calculation of the HEDs for these studies scientifically supported and clearly described? Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

The SAB finds that the selection of convulsions as the endpoint to represent nervous system hazard for RDX is clearly described. The evidence indicates that convulsions is the most sensitive biologically significant endpoint that has been reasonably and reliably measured. However, the SAB notes that evidence from other seizurogenic compounds with similar modes of action suggests that there are other, generally sub-clinical cognitive and behavioral neurological effects that occur at lower doses. It is likely that such effects also occur for RDX although data to establish this are not currently available. For these other compounds, LOELs for triggering abnormal electrographic patterns occur at doses that are 2-3 times lower than doses causing seizure effects. As such, the SAB agrees that the likely dose range between convulsion and other nervous system effects can be addressed using the uncertainty factor adjustments.

The SAB finds that given the presumption that the Crouse et al. (2006) study is the appropriate choice for the derivation of an RfD, and given EPA's choice of a BMR of 1% for deriving a BMDL from Crouse et al. (2006) by benchmark dose modeling, the POD for convulsions was clearly described and correctly calculated. However, the SAB questions both premises and therefore, a positive response to this charge question should be seen as conditional on its recommendations for the two issues detailed in the overall response to this charge question and in response to charge question 4a. in Section 3.4.1.

The SAB agrees the calculation of the HEDs for these studies is scientifically supported and clearly described. EPA estimated the HED by assuming the equivalent pharmacokinetic potency of equivalent rat and human arterial blood concentrations of RDX. The concentration of RDX as a function of time following dosing was generated using a PBPK model, and the effective concentration was estimated as the AUC of concentration and time. The SAB endorses this approach. The SAB agrees that, given the binding of the parent compound to the $GABA_AR$, a dose metric for the parent compound is appropriate, though it also may be serving as a surrogate if any metabolites that have activity. The AUC is a more reasonable choice than C_{max} to estimate the effective concentration due to the uncertainties in the parameterization of the model for absorption.

The SAB does not agree with EPA's use of a BMR of 1% for benchmark dose modeling of the Crouse et al. (2006) data for convulsions. Based on EPA's *Benchmark Dose Technical Guidance* (U.S.EPA, 2012a), the "standard reporting level "(although not *per* se the default) BMR for quantal data (such as those for the incidence of convulsions) is 10%. In that guidance, EPA suggests conditions that would justify BMR values less than 10%. The typical justification given in the guidance for applying a smaller BMR is consideration of the biological process being modeled. In addition, a 1% BMR is recommended for epidemiological data. However, the guidance also points out that "…if one models below the observable range, one needs to be mindful that the degree of uncertainty in the estimates increases. In such cases, the BMD and BMDL can be compared for excessive divergence. In addition, model uncertainty increases below the range of data." The SAB interprets this guidance (and its own common-sense assessment of dose-response modeling) to imply that the choice of a BMR should primarily be directed by the nature of the data. The original intent of the BMR and the resulting BMD, as given in the guidance document, is that it should correspond to a response "near the low end of the observable range." Thus, the BMR should be close to (although not necessarily within) the observable data. The BMR determines the "distance" between the observable data and the BMD. As indicated in the EPA guidance document, the greater the "distance" between the observable data and the BMD, the greater the statistical uncertainty in the fit of the model at the BMD and, therefore, the greater the difference between the BMD and the BMDL.

Table 1 below presents the Lowest-Observed-Adverse-Effect-Levels (LOAELs) and the percent response for convulsions for the Crouse et al. (2006), and Cholakis et al. (1980) studies that EPA considered for RfD derivation.

Table 1. LOAELs and Percent Response at LOAELs for Crouse et al. (2006) and Cholakis et al. (1980)

Study	n/dose group	LOAEL	Percent response at LOAEL
Crouse et al. (2006)	10 rats/sex/dose – group	8 mg/kg/d	15%
Cholakis et al. (1980)	24-25 pregnant rats/dose group	2 mg/kg/d	4%

In the Crouse study, the response at the LOAEL is 15%, in other words, a BMR of 1% would correspond to a response that is a factor of 15 below the lowest observed response data. While the response at the LOAEL in the Cholakis study is lower, it is closer to a BMR of 5% other than the BMR of 1% proposed by EPA.

EPA's choice of a BMR of 1% for modeling the Crouse et al. (2006) data is based on the severity of the convulsion endpoint and the proximity (dose-wise) of convulsions to lethality. EPA's rationale for this choice is to provide a sufficient margin of safety between these two endpoints.

The SAB agrees that the proximity of these two endpoints is, indeed, a valid source of uncertainty in terms of providing sufficient protection for sensitive human populations. However, the SAB believes that dose-response modeling (including benchmark dose modeling) should focus on the data and what can reasonably be concluded from the data about the dose-response close to the range of the observable data. The SAB believes that uncertainty about the appropriateness of the dose-response data and of the POD derived from those data should be addressed through uncertainty factors and not through unsupported extrapolation of the dose-response data.

Therefore, the SAB makes the following recommendations regarding the choice of a BMR:

1. A departure from the standard BMR of 10% for quantal data is justified based on the frank effect in animals.
2. A BMR of 5% based on Crouse et al. (2006) is more consistent with the observed response at the LOAEL of 15%. A 5% BMR for these data is not so far below the observable data (i.e., < the LOAEL) such that the extrapolation from the LOAEL to the BMD itself becomes a significant source of uncertainty.

Table 2 below presents the BMDs for the Crouse et al. (2006) and Cholakis et al. (1980) studies that would result from BMRs of 1%, 5% and 10%. In addition, as suggested by the EPA benchmark dose guidance, the table presents the BMD/BMDL ratios resulting from each BMR.

Table 2. Comparison of BMDs and BMDLs at different BMRs for Crouse et al. (2006) and Cholakis et al. (1980)

Study	**BMR**	**BMD** (mg/kg/d)	**BMDL** (mg/kg/d)	**BMD/BMDL**
Crouse et al. (2006) LOAEL = 8 mg/kg/d	1%	3.02	0.569	5.3
	5%	5.19	2.66	2.0
	10%	6.60	4.59	1.4
Cholakis et al. (1980) LOAEL = 2 mg/kg/d	1%	0.179	0.123	1.5
	5%	0.915	0.628	1.5
	10%	1.88	1.29	1.5

The calculation of the lower bound on the benchmark dose (BMDL) for convulsions is appropriate and consistent with EPA's Benchmark Dose Guidance. For the parameters specified by EPA (including the choice of a BMR of 1%), the benchmark dose is calculated according to EPA's benchmark dose guidance. The choice of the model from among the available dose-response models is appropriate.

3.3.1.4. Uncertainty Factors for Nervous System Endpoints

Charge Question 3a(iv). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.[2]

EPA applied Benchmark Dose Software models to data from two gavage studies in rats (Crouse et al, 2006 and Cholakis et al., 1980) to derive benchmark dose for a 1% response rate (BMDL01) as a point of departure for effects on the nervous system, following Human Equivalent Dose (HED) adjustment. A third data set (Levine et al. 1983) in rats was evaluated using the NOAEL approach. The toxicological endpoint in all cases was convulsions. EPA applied uncertainty factors to the HEDs to derive the proposed RfD for nervous system effects.

Interspecies Uncertainty Factor (UF_A)
An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to the points of departure (PODs), in this case the human equivalent dose for a 1% response rate, to account for the toxicodynamic and residual toxicokinetic uncertainty in extrapolating from average animal models to average humans not accounted for by the toxicokinetic modeling. This is standard risk assessment practice where an adequate toxicokinetic model was applied to derive a human equivalent dose, and available data are not sufficient to define quantitative toxicodynamic differences between species. The SAB agrees that the UF_A of 3 is appropriate.

Subchronic to Chronic Uncertainty Factor (UF_S)
EPA chose a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure. The SAB expressed some concern about the potential for increased sensitivity (i.e., kindling) resulting from additional doses during longer term exposure. However, the SAB concluded that, should any kindling occur, it would be expected to occur within the timeframe (2 weeks-90 days) of the studies evaluated for dose-response. Further, there does not appear to be a greater response in the animals, as measured by incidence of convulsions, with increased exposure duration of the studies. As EPA notes in the draft assessment, the effect chosen as the endpoint (incidence of convulsions) is more related to dose than duration. Thus, the SAB agrees that a UF_s of 1 is appropriate for this endpoint.

The SAB had concern about part of the EPA's rationale for using a UF_S of 1, namely that "in studies of subchronic or gestational exposure used to derive a POD, effects were seen at lower doses in the studies of shorter duration than in the chronic studies"(p 1-11 in the draft assessment). The three studies used to generate PODs were a gestational study with 14-day exposures (Cholakis et al. 1980), a 2-year dietary study in rats (Levine et al. 1983), and a 13-week gavage study (Crouse et al. 2006). As EPA notes in the discussion of the studies and in Appendix C, differences in the method of dose administration, the physical form of RDX, including particle size, and/or dose matrix in the dietary studies and gavage preparations might influence rate of absorption and internal dose, and may partly explain the differences in neurotoxic symptoms reported in the studies of varying duration, both dietary and gavage. In particular, RDX administered orally as a coarse particle preparation was shown to be more slowly absorbed than as a fine particle preparation (Schneider et al. 1977), thus influencing the kinetics of RDX. In the 13-week dietary study of Cholakis et al. (1980), which had a particle size of about 200 µm, no convulsions were reported at doses of 257 mg/kg-day in male and 276 mg/kg-day in female mice. In the 2-year dietary study by Lish et al. (1984), using the same strain of mice, but a smaller particle size (about 66 µm), one convulsion was noted in the 35 mg/kg-day males and in the females in the high dose group (175 mg/kg-day for 11-weeks, followed by lowered dose of 100 mg/kg-day for the remaining duration of the study). In making comparisons of the toxicity of RDX after different durations of exposure, these factors (e.g., particle size, dosing method, and dose matrix) that are known to influence rate of gastrointestinal absorption and/or bioavailability, should be addressed where possible. Note that the test material used in the key study of Crouse et al. (2006), although noted to be of higher purity than most other studies, was not characterized with respect to particle size.

<u>LOAEL to NOAEL Uncertainty Factor (UF_L)</u>

The UF_L is meant to account for uncertainties in extrapolating from a LOAEL to a NOAEL when estimating an RfD. EPA applied a UF_L of 1 because the BMDL was used as a point of departure in Crouse et al. (2006) and in Cholakis et al. (1980), and a NOAEL was used as the point of departure in Levine et al. (1983). Thus, no extrapolation from a LOAEL to a NOAEL was needed. This is standard risk assessment practice and the Panel agrees that this choice is appropriate.

<u>Database Uncertainty Factor (UF_D)</u>

The EPA applied a UF_D of 3 in developing an RfD based on neurotoxicity to help account for database deficiencies. The SAB is concerned that there is limited information available to understand developmental neurotoxicity of RDX. Transplacental and lactational transfer of RDX in rodents has been observed (Hess-Ruth et al., 2007), and therefore, there is potential exposure to the developing fetus and infant from maternal exposure. It is worth noting that Hess-Ruth et al. (2007) concluded that developmental neurotoxicity studies should be conducted for RDX, but apparently this has not been done. EPA noted that the two-generation reproductive and developmental toxicity study of Cholakis et al. (1980) did not report effects in the offspring at doses lower than maternally toxic doses. However, the study only looked at histopathology of the F2 pups at weaning. This study did not assess developmental neurotoxicity in the offspring. The draft assessment indicates that the existing literature did not demonstrate early lifestage as a sensitive subpopulation, but this was not fully evaluated in animal studies and cannot be evaluated with the available human data. There was one case report involving one child poisoned by RDX, but this one case study does not provide evidence regarding the influence of age at exposure on toxicity.

RDX interferes with neurotransmission by binding at the $GABA_AR$, and acting as an antagonist inhibiting GABAergic neurotransmission. GABA is a major inhibitory neurotransmitter in the adult brain. However, GABAergic systems play another role in vertebrate brain development acting as an excitatory neurotrophic factor contributing to processes involved in neurodevelopment (see for example Rivera et al. 1999; Kim et al. 2012). Lead (Pb), a potent developmental neurotoxicant, acts at least partly by inhibiting the GABAergic system during development in zebrafish (Wirbisky et al. 2014) and in mammals (reviewed in Neal and Guilarte, 2013). There is evidence that exposure of early postnatal rodent hippocampal slices to a GABA antagonist (bicuculline) reduces GABAergic neuroactivity, affects the regulation of GABAergic inhibitory synapses and increases their density in the hippocampus (Marty et al. 2000). The hippocampus is involved in seizure development in humans with epilepsy, so these results seem pertinent. There is evidence that drugs that act through the $GABA_AR$ as GABA agonists can also cause neurodevelopmental disorders (see for example the review by Creeley, 2016). These lines of evidence point to potential window(s) of susceptibility in the developing brain to chemicals interfering with GABAergic systems.

Additional evidence prompting concern for developmental neurotoxicity is found in the section of the draft assessment on the mode of action of RDX neurotoxicity. The draft assessment cites a study (Zhang and Pan, 2009) reporting that RDX upregulates 3 microRNAs that affect brain-derived neurotrophic factor (BDNF) in the brains of mice fed 5 mg RDX/kg diet (estimated doses 0.75 to 1.5 mg/kg-day; Bannon et al, 2009). As EPA notes, BDNF is a member of the neurotrophin family of growth factors, and promotes the survival and differentiation of existing and new neurons. BDNF is important for brain development. Disrupting the regulation of BDNF could result in developmental deficiencies in the brain if the perturbation of this pathway were strong enough. This provides additional indirect evidence to generate concern for potential developmental neurotoxicity of RDX.

The SAB recognizes that EPA chose to model a BMDL01 rather than a BMDL10 because of the severity of convulsions as an endpoint. Choosing a lower BMR from a study in adult animals does not, in and of itself, account for the potential of widely different toxicodynamics by age at exposure. EPA does not discuss the role of GABAergic systems in neurodevelopment and the potential for interference with this system by RDX (or other compounds with similar molecular mechanisms) to induce developmental neurotoxicity, an omission that should be rectified. Until there are adequate developmental neurotoxicity studies on this compound, the potential for developmental neurotoxicity as an outcome of RDX exposure remains a data gap.

In the reproductive toxicity study of Cholakis et al. (1980), dosing pregnant dams with RDX recorded incidence data for convulsions that resulted in a 5-fold lower POD than the subchronic study of Crouse et al. (2006), and the candidate RfD. This may indicate that pregnancy is a sensitive window for the adult for neurotoxicity. The EPA noted limitations in the Cholakis et al. data in terms of quantifying the dose-response relationship relative to the Crouse study that was chosen as the basis of the proposed RfD value. These limitations included a lower purity test compound, a 14- day dosing regimen rather than 90 days, and three widely spaced (order of magnitude) dose groupings versus 5 tightly spaced dose groupings in Crouse et al. (2006), all of which impact the accuracy of a POD. The SAB recognizes that these study limitations increase uncertainty in the RfD based on the Cholakis et al. (1980) study. However, the SAB also notes that Cholakis et al. (1980) observed convulsions in a pregnant dam at a dose (2 mg/kg-day) lower than the LOAEL in the Crouse study (8 mg/kg-day) in nonpregnant female rats. Further, in the Angerhofer et al. (1986) teratology study, one death was reported in the dams at 2 mg/kg-day and one death at 6 mg/kg-day, although the authors did not report whether convulsive symptoms occurred prior to death. EPA assessed the LD01 for lethality reported in the studies and noted that the estimates overlay the BMDL01 for convulsions. Thus, mortality occurs in the same dose range as convulsions. This finding provides additional support for using a UF_D of 10 rather than 3.

Finally, there are no studies evaluating incidence of neurological effects that are less severe than convulsions. This data gap forced the EPA to rely on incidence data for convulsions, a frank effect, as a basis for the proposed RfD for RDX. Reliance on a severe effect as the basis for an RfD is unusual. Therefore, EPA should re-consider the database uncertainty factor.

Given the unknown potential for neurodevelopmental toxicity of RDX through interference with GABAergic systems and other pathways, the proximity of the BMDL01 for convulsions to LD01 for lethality, and the lack of incidence data on less severe neurotoxic effects of RDX, the SAB recommends that EPA use a UF_D of 10 rather than 3.

Intraspecies Uncertainty Factor (UF_H)

EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamics variability in the human population. Although a PBPK model was used to extrapolate from the animal internal dose (AUC of RDX in arterial blood) to a human equivalent dose, EPA noted that not enough toxicokinetic data were available from human studies to quantify differences among humans.

The SAB agrees that the UF_H needs to account for both toxicodynamic and toxicokinetic variability among humans, and that not enough data were available to quantitate toxicokinetic or toxicodynamic differences among humans. EPA used the standard default UF_H of 10, which is typically viewed as a composite of a half-log for toxicokinetic differences and a half-log for toxicodynamics differences. Toxicokinetics differences among humans can be related to age, pregnancy, illness, medication use, other chemical exposures, and so on. In the absence of adequate toxicokinetic data to model the range of differences among humans, such differences must be accounted for by including a default toxicokinetic component in the UF_H. The default toxicodynamics portion of the UF_H accounts for differences in target tissue or receptor-mediated response across humans. The SAB agrees with the use of a UF_H of 10.

Recommendations

- EPA should consider applying a UF_D of 10 to account for data gaps for developmental neurotoxicity, lack of incidence data for less severe effects, and the proximity of BMDL01s for convulsions to LD01s for lethality

3.3.1.5. Nervous System-specific Reference Dose

Charge Question 3a(v). Is the organ/system- specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

With regard to the RfD for nervous system effects, the dose derived from the neurotoxicity assessment, especially for convulsions as the critical endpoint, is not supported scientifically as the RfD did not capture all of the potential adverse outcomes or their severity. Convulsions or seizures represent an “all-or-none” quantal phenomenon, with attributes of a steep dose-response relationship for convulsion endpoints. Therefore, the draft assessment should utilize the NOAEL and data from Cholakis et al. (1980) as the primary basis for the RfD in combination with the dose-response data of Crouse et al. (2006). The lower RfD from the observations in the former study (see response to charge question 4a) is judged to be more conservative and indicated in view of the severity of the outcomes (i.e., convulsions) to RDX exposure.

Overall, the conclusion that the available data in humans and animals support a convulsant or proconvulsant neurotoxicity for RDX, possibly through $GABA_AR$ blocking mode of action, is warranted, but the proposed nervous system-specific reference dose is not acceptable. Additional dose specifications should be considered to provide a more reliable dose-response relationship for convulsant and related neurotoxic effects of RDX. In addition, RDX should be considered for the classification as a *potential convulsant or proconvulsant to humans.*

3.3.2. Kidney and Other Urogenital System Effects

3.3.2.1. Kidney and Other Urogenital System Hazard (Sections 1.2.2, 1.3.1)

Charge Question 3b(i). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

The available human, animal, and mechanistic studies support the conclusion that kidney and other urogenital system toxicity are a potential human hazard of RDX exposure. However, this conclusion is primarily supported by animal data, whereas available human studies that implicate the kidney as a potential target of RDX are sparse and only identify transient renal effects following acute human exposure. There are no reports of prostatic effects of RDX in humans and no pertinent mechanistic data regarding RDX effects on the kidney and urogenital system.

All hazards to the kidney and urogenital system are adequately assessed and described in the draft assessment, with the exception of the description of inflammatory changes in the rat prostate. The description in the draft assessment of these prostatic inflammatory changes should include not only suppurative inflammation, but also chronic inflammation and the variability and uncertainty in the classification of prostatic inflammation.

The selection of suppurative prostatitis as the endpoint (“surrogate marker”) to represent this hazard is clearly described in the draft assessment, but not scientifically supported because of various uncertainties that are associated with the hazard, including the following:

- There is no known biological basis for using this suppurative prostatitis as a surrogate marker for renal and other urogenital (GU) effects.

- Uncertainty about the direct association between suppurative prostatitis and the toxic renal effects observed in male rats in the Levine et al. (1983) study. A strong association between kidney lesions (papillary necrosis, pyelonephritis, and peri-renal peritonitis) and suppurative inflammation in the prostate was observed only in males in the highest dose group; there were no such renal changes in the lower dose groups (except in one male animal in the 8.0 mg/kg-day group), while suppurative prostatitis occurred at the two next highest does (8.0 and 1.5 mg/kg-day). The renal lesions were considered primary effects of RDX in the draft assessment, while the prostatitis was considered secondary to the renal effects; the SAB concurs with this notion.

- Uncertainties about the diagnosis of suppurative inflammation:

    (a) Suppurative and chronic inflammation are part of a continuum and diagnostic criteria may have varied over time and among pathologists. Prostatic inflammation found in aged rats is divided into several subtypes, only one of which is suppurative inflammation. Other categories include subacute inflammation, chronic-active inflammation, and micro-abscesses. Reference is made in the draft assessment to a paper by Suwa et al. (2001) about background pathology in the prostate of 1,768 control F344 rats allowed to live for up to 2.4 years. This paper was the basis for the EPA conclusion that inflammation in the control group of the study by Levine et al. (1983) was unusually low for this strain of rats. However, in the paper by Suwa et al. (2001), all types of inflammation are combined, and 70.4% of these rats had inflammation mostly confined to the dorsolateral prostate and graded as mild. No data were provided by Suwa et al. on suppurative inflammation.

    (b) Combining all types of prostate inflammation in the 24-month groups of the Levine et al. (1983) study yields similar incidences among all groups, with the exception of the highest dose group. The prostatitis incidences in the control and the three lowest dose groups were about 40% lower than the incidences reported by Suwa et al. (2001) for aged F344 rats in NTP studies; the lower incidences may be a reflection of the manner of histopathologic examination (see point c) below).

    In the Levine *et al*. study, 23/55 control rats (42%) had prostatic inflammation of any type (chronic and suppurative inflammation) and the incidence in the 0.3, 1.5, and 8.0 mg/kg-day dose groups was 20/55 (36%), 22/52 (44%), and 23/55 (42%), respectively; these lesions were mostly graded as minimal to mild. Two of the 3 rats that died between 6 and 12 months in the 1.5 mg/kg-day group had (sub)acute prostatic inflammation, and (sub)acute prostatitis was also diagnosed in 1 of 10 rats in the 1.5 mg/kg-day dose group and 2 of 10 rats of the 8.0 mg/kg-day dose group that were sacrificed at the scheduled 12-month interim time point.

    By contrast, 51 of 55 rats in the high dose (40 mg/kg-day) group died before the end of the two-year study and 38 of these 51 rats had prostatic inflammation. Prostatic inflammation (chronic type) was found in 1 of 5 rats in the high dose group that died before 6 months and in 18 of 19 (95%) rats in the high dose group that died between 6 and 12 months. Suppurative inflammation was found in 18 of 27 (67%) rats that died between 12 and 24 months in the high dose group and chronic inflammation occurred in 1 of these 27 rats. One of the 4 high dose rats that lived until the end of the study had minimal chronic inflammation of the prostate; no suppurative prostatic lesions were found in these 4 rats. Thus, the total incidence of prostatic inflammation of any type in the high dose group of the 24-month study was 39 of 55 rats (71%). Twenty of the 31 rats (65%) that died after the 12-month time-point (including the 4 that survived until the end of study) had prostatic inflammation, which was suppurative in nature in 18 rats and of the chronic type in 2 rats.

In the Levine et al. (1983) study, there was a shift from chronic inflammation to suppurative inflammation in the prostate with increasing RDX dose at 1.5 mg/kg-day and higher. This shift is statistically significant if tested using a Chi-squared test, with categories set for no lesions, chronic inflammation, and suppurative inflammation across all treatment groups ($P < 0.0001$) (prostatic inflammation was scored by Levine et al. as either chronic or suppurative). The shift was almost complete in the 31 rats that died in the 40 mg/kg-day group after 12 months on study, as only two animals had (minimal) chronic inflammation and 18 had suppurative inflammation. Although this analysis ideally should have taken into account mortality differences, the Levine study does not contain data that allow one to do this, as pointed out in the draft assessment.

(c) The description of the methods used for histopathological evaluations lacked detail in Levine et al. (1983), which is an important issue given the large variation known for inflammation among the four prostate lobes, based on NTP data of aged F344 rats. The fact that the incidence of prostatic inflammation in the control group of the Levine *et al*. study was 40% lower than the range of inflammation incidences found in the dorsolateral prostate by Suwa et al. (2001) would suggest that some or many of the prostates examined by Levine et al. were ventral lobes, which have a low inflammation incidence (4-12%), according to Suwa et al. (2001). Suwa et al. indicated that there was considerable variation in which lobes were present and examined in the NTP studies they reviewed, suggesting that some of the study-to-study variation in the incidence of prostatic inflammation may be due to variations in which prostate lobes were examined.

(d) There was no peer review or pathology working group review of the Levine et al. (1983) renal, bladder, and prostate pathology data, as was done for the liver lesions in female mice in Lish et al. (1984).

(e) There may have been potential effects of the high prevalence of fighting in highest dose rats and resultant individual housing of all males in the Levine et al. (1983) study. There is evidence in the literature that fighting may cause urogenital infections in male rats (Creasy et al. 2012). Because of this fighting, all males in the highest dose group were individually housed from 30-40 weeks into the study, which introduced another factor different from the other treatment groups that may have affected the animals in the 40 mg/kg-day group in uncontrolled ways.

Recommendations

- The SAB recommends suppurative prostatitis not be used as a “surrogate marker” of renal and overall urogenital effects, but instead, be considered as a separate effect (see also Section 3.3.2.5.).
- Improve the description and analysis of prostatitis to include both chronic and suppurative inflammation.

- Improve the description of the various uncertainties regarding the Levine et al. (1983) rat study.

3.3.2.2. Kidney and other urogenital system-specific toxicity values (Section 2.1.1).

Charge Question 3.b(ii). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

The selection of the Levine et al. (1983) study that found kidney and other urogenital system effects is clearly described, but not entirely supported scientifically.

While the renal and bladder effects found in male rats in the high dose group of the study by Levine et al. (1983) were treatment-related and were the most likely cause of mortality in this group, the effects on the prostate were less straightforward [see also response to charge question 3b(v)].

One male in the lowest dose group (0.3 mg/kg-day) of 55 rats had renal papillary necrosis, but no other animals in the control or 1.5 and 8.0 mg/kg-day dose groups had this lesion. By contrast, it was found in 33 of 50 male animals in the high dose group (40 mg/kg-day) (15 of 19 rats that died before the 12-month interim sacrifice and 18 of 27 rats that died between 12 and 24 months – the lesion was not found in the 4 survivors at 24 months). Hemorrhagic/suppurative cystitis was found in 35 of 50 male rats of the high dose group, but in only one or two males per group in the lower dose groups and in none of the controls. These renal and bladder lesions tended to be more severe after 12 months on study than in rats examined at the six- and twelve-month interim necropsies. Prostatic effects, namely a significant shift from chronic to suppurative inflammation, were seen at doses of 1.5 mg/kg-day and above and the overall incidence of prostatic inflammation was significantly increased in male rats of the high dose group (40 mg/kg-day).

However, the Levine et al. (1983) study was not the only animal study that found effects on the kidney. Renal medullary mineralization was reported by Martin and Hart (1974) in 3 of 4 male and 3 of 4 female Cynomolgus monkeys in the highest dose group tested (10 mg/kg-day), but not at lower RDX doses or in controls. Cortical tubular nephrosis was found in 4 of 10 male and 1 of 10 female B6C3F1 mice at a very high RDX dose of 320 mg/kg-day, while this lesion was not present in control male or female mice (Cholakis et al. 1980). Both studies were of 90 days duration and the renal effects were minimal to moderate in severity and not or only marginally statistically significant. Cholakis et al. (1980) did not find any renal lesions in male F344 rats and only minimal microcalculi (mineralization) in 1 of 10 female rats exposed to 40 mg/kg-day RDX via the diet for 90 days. In a two-generation study by Cholakis et al. (1980), renal cortical cysts, but no other renal lesions, were found in both control and treated CD (Sprague Dawley) rats.

Another 90-day study in F344 rats used lower doses by gavage and found no evidence of any treatment-related renal effects in males, while minimal-to-mild microconcretions (mineralization) were found in 4 of 10 females that were administered RDX at a dose of 15 mg/kg-day and in 7 of 10 control females (Crouse et al. 2006). Levine et al. (1981a) found frequent microconcretions (mineralization) in female, but not in male F344 rats, administered

RDX via the diet for 90 days; control rats were without evidence of a treatment related effect. Levine et al. (1981a) also found nephropathy in both sexes, the incidence of which was reduced in the highest dose group (100 mg/kg-day); this reduction was significant in males but not in females. No renal toxicity was found in a 90-day dog study with dietary RDX doses up to 10 mg/kg-day (Hart et al. 1974). The only report of a lesion in the prostate came from the 90-day study by Crouse et al. (2006) in F344 rats administered RDX by gavage at a dose of 15 mg/kg-day; 1 of 8 males had mild subacute inflammation in the prostate, while no prostate lesions were found in 10 controls. There were no prostate lesions found in any of the other 90-day studies mentioned above. In the 24-month study by Lish et al. (1984), a high frequency of cytoplasmic vacuoles in the renal tubular epithelium, with minimal-to-mild severity, was observed in male B6C3F1 mice at the 6, 12, and 24-month time points; the male control group was an exception with only a 10% incidence of these cytoplasmic vacuoles at the 6-month interim sacrifice. Female mice had a low incidence of this renal change and this alteration was not reported in any of the other studies mentioned above.

In aggregate, mild toxic effects of RDX exposure on the kidney were found in some species, but not others, and in some studies in both sexes but in other studies only in male or female animals. Of note some of these effects (mineralization) occurred in a small study with non-human primates, whereas some rodent studies did not find evidence of renal toxicity. Only in the chronic study of Levine et al. (1983) were severe toxic effects on the kidney found and they only occurred in males at the highest dose (40 mg/kg-day); bladder toxicity also occurred in this treatment group, whereas effects on the prostate occurred at doses of 1.5 mg/kg-day and above.

The marked gender difference in the renal toxicity due to RDX exposure found for rats by Levine et al. (1983) is not discussed in the draft assessment. However, there is precedent for a toxic chemical causing renal papillary necrosis selectively in male, but not female, F344 rats (Neal et al. 2003) and several drugs are well known for gender-specificity in their ability to cause renal papillary necrosis (Bach and Nguyen, 1998; Brix, 2002).

Recommendations

- Improve the description and analysis of renal effects observed in studies other than those reported by Levine et al. (1983).
- Briefly include a discussion of the marked sex difference in the renal toxicity in rats due to RDX exposure.

3.3.2.3. Points of Departure for Kidney and Other Urogenital System Endpoints (Section 2.1.2)

Charge Question 3b(iii). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

The SAB strongly recommends that suppurative prostatitis not be regarded as a surrogate marker for kidney and other urogenital system endpoints [see responses to charge questions 3b(i), 3b(ii), and 3b(v)]. If suppurative prostatitis is considered as a stand-alone endpoint, separate from kidney and other urogenital system endpoints, the calculation of both the POD and HED are scientifically supported and clearly described.

EPA's software BMDS was used to fit ten dose-response models to the data from Levine et al. (1983), and all models provided reasonable fits according to standard goodness-of-fit measures. Using a BMR of 10%, corresponding estimated BMDs for the models ranged from 1.67 to 10.8 mg/kg-day, with associated BMDLs ranging from 0.469 to 8.58 mg/kg-day. BMDLs from the ten models differ by more than threefold, so the lowest BMDL was selected, consistent with EPA guidance. The selected log-probit model has an estimated BMD of 1.67 mg/kg-day, which is within the range of study doses, thus obviating any issues of inappropriate extrapolation. The suppurative prostatitis POD for rats was determined to be 0.469 mg/kg-day.

Three methods were used to calculate the HED corresponding to the BMDL— one based on allometric scaling ($BW^{3/4}$), another based on equivalent RDX serum AUCs in rats and humans at steady state, and a third based on equivalent RDX maximum serum concentrations in rats and humans after dosing. The methods for these calculations are clearly explained. The quality of data used for PBPK modeling are variable with respect to toxicity, but the resulting HED appear appropriate, with preference given to the AUC-based derivation. The alternative approach of allometric scaling was believed to introduce too many uncertainties.

3.3.2.4. Uncertainty Factors for Kidney and Other Urogenital System Endpoints

Charge Question 3b(iv). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

The draft assessment used suppurative prostatitis in a 2-year study in male rats (Levine et al., 1983) as a surrogate marker for the entirety of observed adverse effects of RDX exposure on the kidney and urogenital system. BMDS models were used to fit the data from Levine et al (1983) using a 10% benchmark response rate (BMR). The human equivalent dose (HED) for the POD was calculated based on three methods. Uncertainty factors were then applied to the BMDL10 HED to derive an RfD specifically for the kidney and urogenital system.

The SAB recommends that separate RfDs be derived for the kidney and urogenital system based on findings of renal papillary necrosis and associated renal inflammation, and for suppurative prostatitis. This distinction designates the male accessory sex glands as a separate organ system (see response to charge questions 3b(i)), and challenges EPA's selection of suppurative prostatitis as a surrogate marker for the entirety of adverse effects on the kidney and urogenital system. This recommendation is keeping with the fact that there is no known mechanistic link between suppurative prostatitis and renal papillary necrosis or adverse effects on renal function. Therefore, the charge question regarding the application of uncertainty factors can only be answered at this time for suppurative prostatitis, since an RfD has not been derived for renal papillary necrosis. Thus, the comments on the application of uncertainty factors are only relevant for the RfD derived based on suppurative prostatitis.

Intraspecies Uncertainty Factor (UF_H)

EPA applied an intraspecies uncertainty factor of 10 to account for toxicokinetic and toxicodynamic variability in the human population, which is standard default risk assessment practice. Although a PBPK model was used to extrapolate from the animal internal dose (AUC of RDX in arterial blood) to a human equivalent dose, EPA notes that not enough toxicokinetics data were available to quantify the differences among humans.

The SAB agrees that the UF_H needs to account for both toxicodynamic and toxicokinetic variability among humans, and that not enough toxicokinetic or toxicodynamic data were available to quantify differences among humans. EPA used the standard default UF_H of 10, which is typically viewed as a composite of a half-log for toxicokinetic differences and a half-log for toxicodynamic differences. Toxicokinetic differences among humans can be related to age, pregnancy, illness, medication use, other chemical exposures, and so on. In the absence of adequate toxicokinetic data to model the range of differences among humans, such differences must be accounted for by including a default toxicokinetic component in the UF_H. The default toxicodynamics portion of the UF_H accounts for differences in target tissue or receptor-mediated response across humans. The SAB agrees with the use of a UF_H of 10.

Interspecies Uncertainty Factor (UF_A)

An interspecies uncertainty factor, UF_A, of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to the point of departure to account for the remaining toxicodynamic and residual toxicokinetic uncertainty not accounted for in the toxicokinetic modeling. This is standard risk assessment practice where an adequate toxicokinetic model was applied to derive a human equivalent dose, and available data are not sufficient to define quantitative toxicodynamic differences between species. The SAB agrees with the application of a UF_A of 3.

Subchronic to Chronic Uncertainty Factor (UF_S)

The draft assessment used a UF_S of 1 to extrapolate from a subchronic experimental exposure duration to chronic exposure. The Levine et al (1983) study was a chronic duration exposure study, and thus no extrapolation factor is needed. This SAB agrees that this is appropriate.

LOAEL to NOAEL Uncertainty Factor (UF_L)

The UF_L is meant to account for uncertainties in extrapolating from a Lowest-Observed-Effect Level to a NOAEL when estimating an RfD. A UF_L of 1 was applied because the BMDL was used as a point of departure. Thus, there is no need to extrapolate from a LOAEL to estimate a NOAEL. This is standard risk assessment practice, and the SAB agrees that this is appropriate.

Database Uncertainty Factor (UF_D)

The assessment applied a UF_D of 3 in developing an RfD for suppurative prostatitis. The draft assessment notes that additional studies on neurotoxicity may provide a more sensitive endpoint to use as the basis of an RfD. Thus, a UF_D of 3 was applied across all points of departure, regardless of endpoint. In evaluating the RfD based on neurotoxicity, the SAB recommends using a UF_D of 10 rather than 3 due to database limitations. This recommendation would be relevant to an RfD for suppurative prostatitis if such an RfD were to be the basis of the overall RfD. However, if the RfD for suppurative prostatitis were only to be used specifically in a hazard index approach for this target, then an organ-specific UF_D may be appropriate.

Recommendations

- The SAB recommends that EPA develop or cite documentation for the use of organ-specific reference values for individual chemicals; specifically, including how these would be used in assessing the combined noncancer health impacts of multiple agents acting at a common site in cumulative risk assessments.

- The SAB recommends that a separate RfD be derived for renal papillary necrosis and the associated renal inflammation for the kidney and urogenital system and that the male accessory sex glands be designated as a separate organ system with a separate RfD derived for suppurative prostatitis.

3.3.2.5. ***Kidney and other urogenital system-specific reference dose*** *(Section 2.1.4).*

Charge Question 3.b.v. Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

The organ/system-specific reference dose derived for kidney and other urogenital system effects is not sufficiently supported scientifically or clearly characterized. The selection of suppurative inflammation of the prostate observed in the Levine *et al*. (1983) study as a “surrogate marker” of the observed renal and urogenital system effects is not justified for derivation of a reference dose (RfD) [see response to Charge Question 3b(i)].

Separate RfDs should be derived for renal papillary necrosis and the associated renal inflammation and for suppurative prostatitis. Separate candidate RfDs could be considered for other, milder renal effects (tubular nephrosis, epithelial vacuolization, and mineralization) found in subchronic studies in mice, rats, and monkeys. However, the SAB concludes that the available data are not consistent enough across species, doses, sex, or time points to recommend such an approach.

3.3.3. Developmental and Reproductive System Effects

3.3.3.1. Developmental and Reproductive System Hazard

Charge Question 3c(i). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

The SAB’s response to Charge Question 3c(i) is subdivided into three components:

No Evidence of Male Reproductive Effects:

The SAB disagrees with the conclusion in the draft assessment that there is suggestive evidence of male reproductive effects associated with RDX exposure. As outlined in detail in the SAB response to Question 3c(ii), the available animal evidence is too weak to support this statement. There is no human evidence indicating male reproductive toxicity; no human studies have focused on this question and there were no incidental reports of reproductive effects following RDX exposures. Furthermore, the mechanisms of action of RDX do not provide reasons to expect male reproductive toxicity.

Conclusions as to whether Developmental Effects are a Human Hazard of RDX Exposure:

The SAB concludes that there are sufficient available data to draw the conclusion that RDX does not pose a teratogenic risk to humans based on animal data in which none of the developmental studies conducted in the rat and rabbit reported a teratogenic outcome, despite the use of doses that were high enough to occasionally produce maternal toxicity. The SAB encourages EPA to include this important piece of information for the overall risk assessment in the draft assessment. Additionally, the SAB agrees that conclusions cannot be drawn regarding other forms of developmental toxicity, which occurred only at maternally toxic dose levels.

The developmental toxicity observed was typical of findings associated with maternal toxicity and occurred at maternally toxic dose levels. It is generally understood that maternal toxicity, evidenced by body weight loss or reductions in body weight gain and/or decreases in food consumption, can contribute to developmental toxicity of the fetus in animal models. Developmental toxicity associated with maternal toxicity typically manifests as fetal weight reductions, increases in post-implantation loss (i.e., embryo/fetal death), and increases in the incidence of certain fetal skeletal variations. There is recognition within the scientific community of the possible effects on the fetus from maternal toxicity in common animal models [Carney and Kimmel, 2007; Rogers et al. 2005]. This concept was the primary topic discussed in an International Life Sciences Institute-Health and Environmental Sciences Institute (ILSI-HESI) sponsored working group, and the proceedings have been published [Beyer et al. 2011]. The findings in the RDX developmental toxicity studies of increased post-implantation loss, decreased fetal body weight and fetal skeletal variations are those considered typically associated with maternal toxicity and occurred at maternal toxic dose levels.

In an embryo fetal developmental (EFD) toxicity study in F344 rats maternal toxicity (mortality up to 31%) and developmental toxicity (reduced fetal body weights and increased resorptions) occurred at 20 mg/kg-day (Cholakis et al. 1980). In SD rats at 20 mg/kg-day, there was maternal toxicity and increased resorptions (Angerhofer et al. 1986). No teratogenicity occurred at these doses or lower doses in either rat strain. Treatment in both of these studies starts on gestation day 6, while implantation is still in progress and ends on gestation day 15, prior to the closure of the hard palate. A longer dosage period as suggested for all current EPA and OECD guidelines, may have yielded more fetal toxicity, especially an effect on fetal weight.

The only two generational study identified reported decreases in offspring survival (including stillborn pups and postnatal deaths through the age of weaning) following a clearly maternally toxic dose of 50 mg/kg-day, administered in the diet and adjusted approximately weekly (Cholakis et al. 1980). Lower doses were not toxic to the dams or offspring.

Rabbits evaluated in an EFD toxicity study dosed on days 6 to 29 of gestation appear to be less sensitive than rats as exposures up to 20 mg/kg-day did not produce any maternal or embryo/fetal toxicity (Cholakis et al. 1980). The conclusion of apparent less sensitivity is difficult to support without knowing if exposure in the rabbit was equivalent to or more than that observed in the rat. Effective doses scaled by allometric scaling suggest that the rabbit probably experienced a higher dose than the 20 mg/kg-day in the rat. The rabbit embryo fetal development study at 0.2, 2 and 20 mg/kg-day showed fetal malformations with a low incidence at the 20 mg/kg-day dose and these changes were not present in control fetuses or seen at lower dose levels. The incidences ranged from 1 to 3 % and included a variety of malformations with no apparent biological relatedness, none of which were statistically significant. These data are difficult to put in context without a robust historical control database, systemic exposure levels and knowledge of the litter size. Additionally, the maternal rabbits at 20 mg/kg-day gained less weight, so there was some maternal toxicity at the high dose tested. However, this is not necessarily an associated factor for the possible malformations. The report states that the findings were not statistically significant and thus RDX was not considered specifically teratogenic in rabbits.

Other Hazards to Human Reproductive and Developmental Outcome:

Based on *in vitro* data, the SAB concludes that the mechanistic-based hazard demonstrating RDX inhibits GABAergic neurons presents a potential neurodevelopmental hazard that was not adequately addressed in the draft assessment. Several lines of evidence point to potential window(s) of susceptibility in the developing brain to chemicals interfering with GABAergic systems (see database uncertainty factor discussion in Section 3.3.1.4).

A pilot developmental neurotoxicity study was conducted in rats which demonstrated significant accumulation of RDX in the immature brain of offspring and in the milk from dams treated with 6 mg/kg-day during gestation (Hess-Ruth, 2007). This dose level induced convulsions in adult animals. There were approximately equal concentrations (µg/mL) in maternal blood and milk, and higher levels in younger postnatal day (PND) 1 pup brains compared to PND 10. A stated conclusion from this report was that further studies evaluating neurotoxicity and developmental effects of RDX should be conducted. It does not appear that a follow up study was conducted, thus no definitive assessment of potential developmental neurotoxicity in rats can be completed to inform risk for humans. Regardless, the SAB encourages the inclusion of a description of the potential mechanistic-based hazard in the draft assessment based on the reported mechanism to inhibit GABAergic neurons and the knowledge that RDX is present in the brain of developing rats during gestation and lactation.

3.3.3.2. Reproductive System-Specific Toxicity Values

Charge Question 3c(ii). Is the selection of the Lish et al. (1984) study that describes male reproductive system effects scientifically supported and clearly described?

In consideration of all evidence, the SAB does not agree that the selection of Lish et al. (1984) for male reproductive effects is supported scientifically, and offers further suggestions on how to describe the data.

The SAB determines that the suggestive evidence of male reproductive effects provided by Lish et al. (1984), based on testicular degeneration in male mice exposed to RDX in their diet for 24 months, is weak, unsupported by other endpoints in that study showing no effect, complicated by the age of the mice and general toxicity of the RDX dose, and contradicted by most other studies.

In the study of Lish et al. (1984), the 10% and 11% incidence of testicular degeneration observed at doses of 35 and 108 mg/kg-day was not considered to be statistically significant. Also no histological changes were observed at 6 or 12 months of study, times much longer than the 1.4-month duration of spermatogenesis in mice. Furthermore, significant decreases in testis weight, which should have been observed if there were appreciable degeneration, were not observed.

The validity of 24-month chronic toxicity studies to evaluate male reproductive toxicity in rodents is questionable because of the loss of testicular function that occurs with aging in both rats and mice. In rats, the manifestations of aging in 2-year old animals include a high incidence of interstitial cell tumors (Cohen 1978), declines in sperm production (Wang et al. 1993; Johnson & Neaves, 1983), reduced gonadotropin levels (Bruni et al. 1977), and reduced testosterone production due to aging of Leydig cells (Chen et al. 2002). In 2-year old mice, reductions in sperm counts and hormone levels were also observed (Bronson & Desjardins, 1977; Gosden et al. 1982), and in addition there were reductions in the numbers of stem spermatogonia, the loss of functional ability of theses stem cells, and failure of the somatic environment to support spermatogonial differentiation (Suzuki & Withers, 1978; Zhang et al. 2006). Effects observed in rodents exposed to a potential reproductive toxicant in a 2-year chronic toxicity study may represent the combined effects of toxicant and aging, and not the result of prolonged treatment. Regarding the application of such results to the human population, effects of a toxicant on sperm production in aged men is not considered an important reproductive risk, as such men rarely desire to have children.

In addition, the indication of an effect of RDX on spermatogenesis suggested by Lish et al. (1984) is generally not supported by other studies (Table 3). In particular, Cholakis et al. (1980), using the same mouse strain, did not find any significant effects of RDX doses up to 320 mg/kg-day in a 3-month subchronic study. Although the RDX used by Cholakis et al. was of larger particle size than that used by Lish et al., which could reduce the uptake of RDX, mortality of the animals in the Cholakis et al. study administered 320 mg/kg-day was equivalent to that observed Lish et al. at 175 mg/kg-day indicating effective uptake of the RDX particles. Since 3-months allows for more than two complete rounds of spermatogenic cell differentiation, this should have been sufficient time at which to detect a toxic effect.

Furthermore, studies in rats indicate little male reproductive toxicity of RDX. In a 2-year chronic study, Hart et al. (1976) found no testicular degeneration or weight loss at doses up to 10 mg/kg-day. Similarly, Levine et al. (1983) found no effects of a dose of 8 mg/kg-day. However, at 40 mg/kg-day there was a significant decline in testis weight (14%) and a significant increase in the percentage of testes showing gem cell degeneration at 12 months of treatment. Although the effect was significant, the fact that there was 30% excess mortality by this time may indicate that the testicular damage was secondary to general toxicity. Data obtained at 24 months were not meaningful since all rats of this strain developed Leydig cell hyperplasia/neoplasms by 2 years of age.

Moreover, three 13-week subchronic studies in rats also failed to indicate significant testicular damage. Levine et al. (1981a,b; 1990) found no significant testicular effects of exposure to doses up to 100 ng/kg-day. Also Cholakis et al. (1980) found no changes in absolute testis weights or histopathological damage to testes at 28 or 40 mg/kg-day. Similarly, Crouse et al. (2006), in the only study using gavage, which had greater potency than dietary administration as indicated by 20-30% mortality at doses of 10-15 mg/kg-day, reported no significant histological effects or changes in absolute testis weights. The additional data of Cholakis et al. (1980) obtained as part of a 2-generational study, did indicate an 18%reduction in proportions of females impregnated by males exposed to RDX at 50 mg/kg-day. While this could reflect at testicular effect, it would also be a behavioral effect or a systemic effect as suggested by the 14% excess mortality in this group.

Finally, the SAB did not find the selection of Lish et al. (1984) to be clearly described, and provided specific comments in Appendix B on the text, tables and figures to improve presentation of data on reproductive and developmental toxicity

Table 3. Summary of Results of 7 Studies of Male Reproductive Toxicity of RDX

Study	**Species**	**Route**	**Significant Effect**	**Doses (**mg/kg-day) **Time** (months)	**Caveats**	**Negative Results** (non-significant considered as negative)
Lish et al. (1984)	Mouse	Diet	None	35 & 108 24 mo.	Mortality∗ (>14%) Age-related effect	No histological change at 6 or 12 mo. The 10-11% incidence in testicular degeneration at 24 mo. was not significant. No decrease in testis weight
Cholakis et al. (1980)	Mouse	Diet	None	40, 80, 160, 320 3 mo.		No histological changes No decrease in testis weight
Levine et al. (1983)	Rat	Diet	40% increase in incidence of germ cell degeneration 14% decrease in testis weight	40 mg/kg-day 12 mo.	Mortality∗ 31% at 12 mo.	No effects at 8 mg/kg-day No effects at 6 months with 40 mg/kg-day No germ cell degeneration at 40 mg/kg-day at 24 mo.
Hart (1976)	Rat	Diet	None	10 mg/kg-day 12 & 24 mo.		No histological changes (24 months) No decrease in testis weight
Cholakis et al. (1980)	Rat	Diet	18% reduction in proportion of females impregnated †	50 mg/kg-day 3 mo.	Mortality∗ 14% Possible behavioral effect	Reduction in impregnation not observed at 16 mg/kg-day No histological changes at 40 mg/kg-day No decreases in testis weight at 28 or 40 mg/kg-day
Levine (1981a,b; 1990)	Rat	Diet	None	10, 30, 100 3 mo.		No histological changes No decreases in testis weight
Crouse et al. (2006)	Rat	Gavage	None	15 mg/kg-day 3 mo.		No histological changes No decreases in testis weight

∗ Excess mortality compared to observed in controls
† Calculated as significant by reviewer using Chi-square test at P=0.004

3.3.3.3. Points of Departure for Reproductive System Endpoints

Charge Question 3c(iii). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

As discussed elsewhere (e.g., in response to questions 3c(i) and 3c(ii), the SAB does not support use of the Lish et al. (1984) study for describing male reproductive system effects. Given that Lish et al. (1984) was the data source for building dose-response models and subsequent derivations such as the POD and HED, the SAB doubts the validity of these derived POD and HED.

The SAB can only respond to the charge question by placing a contingency on its response: assuming the use of testicular degeneration from Lish et al. (1984) as the endpoint, calculation of both the POD and HED are scientifically supported and clearly described.

EPA's software BMDS was used to fit ten dose-response models to the data from Lish et al. (1984), and all models provided reasonable fits according to standard goodness-of-fit measures. Using a BMR of 10%, corresponding estimated BMDs for the models ranged from 56.0 to 97.1 mg/kg-day, with associated BMDLs ranging from 16.3 to 66.1 mg/kg-day. BMDLs from the ten models were not "sufficiently close," so the lowest BMDL was selected, consistent with EPA guidance. The selected log-probit model has estimated BMD of 56 mg/kg-day, which is well within the range of study doses, so there is no concern about inappropriate extrapolation. The testicular degeneration POD for mice was determined to be 16.3 mg/kg-day.

Three methods were used to calculate the HED corresponding to the BMDL— one based on allometric scaling ($BW^{3/4}$), another based on equivalent RDX serum AUCs in mice and humans at steady state, and a third based on equivalent RDX maximum serum concentrations in mice and humans after a dose. The methods for these calculations are clearly explained, as are concerns regarding the PBPK model for mice and the low confidence in the PBPK model for extrapolating from mice to humans. As a result, EPA selected allometric (body weight) scaling to derive the HED for the testicular degeneration POD.

3.3.3.4. Uncertainty Factors for Reproductive System Endpoints.

Charge Question 3c(iv). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

The draft assessment used the data on testicular degeneration in mice from a 2-year dietary study (Lish et al. 1984) as the basis for derivation of the POD. BMDS models were used to fit the incidence data to derive a BMDL for a 10% BMR. Three methods were used to derive an HED from the mouse POD. The draft assessment notes that the toxicokinetic data available for the mouse are not as robust as for the rat, and thus confidence in the use of PBPK modeling to account for interspecies toxicokinetics is low. Rather, the default allometric scaling approach was used to derive the HED by scaling dose by ¾ power of body weight. After adjusting the mouse POD to an HED with this scaling, uncertainty factors were applied to derive an RfD for male reproductive toxicity.

The SAB does not support derivation of a RfD based on male reproductive system effect (see Section 3.3.3.5), and, concludes that an RfD based on testicular degeneration is not supported scientifically. The question of uncertainty factors as applied to the POD is therefore extraneous.

3.3.3.5. Reproductive System-specific Reference Dose.

Charge Question 3c(v). Is the organ/system-specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

The RfD for reproductive effects based on testicular degeneration is clearly described but not scientifically supported. Reasons for this conclusion are provided above in response to question 3c(ii), and briefly summarized below.

Testicular degeneration was reported at terminal sacrifice (24 months) in one 2-year dietary study in mice (Lish et al. 1984). Germ cell degeneration was also observed in a 2-year dietary study in rats (Levine et al. 1983) but only at the 12-month interim sacrifice and not at the 6-month interim or 24-month terminal sacrifice. The SAB noted that testicular histopathology should have been seen at earlier time points (e.g., the 6-month and 12-month interim sacrifices) in Lish et al. (1984), as these exposure durations were several times longer than the 1.4 month duration of spermatogenesis in mice. Further testicular degeneration was not observed in the majority of the dietary and gavage studies in rodents (5 of 7 showed no effect).

Other reproductive effects observed included changes in testicular absolute and relative weight, but these findings were inconsistent across studies. Effects on fertility were noted in a 2-generation reproductive study in CD rats at the high dose (50 mg/kg-day) (Cholakis et al, 1980), but both the male and female rats had decreased weight gain and increased mortality and thus it was difficult to attribute the reduction in fertility to a specific reproductive toxicity effect of RDX. In a dominant lethal assay (Chokakis et al. 1980), decreased rates of pregnancy of untreated females when mated with F0 males treated with 50 mg/kg-day may have been associated with generalized toxicity in the treated males rather than a specific effect of RDX. There were no observations of histological changes in the testis or decreased testicular weight in any of the treated animals in Cholakis et al. (1980).

The Agency provided the BMDS analysis in Appendix D of the Supplemental Document, and clearly described the rationale for deriving the HED and applying the uncertainty factors. However, since the toxicological effect used as the basis of the RfD was testicular degeneration, and this is not supported scientifically, then the RfD is not supported scientifically.

3.3.4. Other Noncancer Hazards

Charge Question 3d. The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal and mechanistic studies support this decision. Are other non-cancer hazards adequately described?

The potential "other non-cancer hazards" from RDX exposure are identified and discussed in Section 1.2.4 and 1.3.1 (liver), and Section 1.2.6 and Appendix C.3.2 (ocular, musculoskeletal, cardiovascular, immune system, gastrointestinal, and hematological) of the draft assessment. In Appendix C.3.2, lines 5-6 it states "Overall, at the present time, the evidence does not support identifying these other systemic effects as human hazards of RDX exposure." In the subsequent paragraphs summarizing the evidence for the other systemic effects, the text does not provide a clear rationale for why the evidence does not support the listed effects as potential human hazards. For example, is it due to insufficient data, inconsistent data, or sufficient data to conclude that these health endpoints are not sensitive endpoints?

In the process of identifying the health hazards of RDX, a conclusion should be made for each hazard endpoint discussed based on the available evidence streams (human, animal, and mechanistic), and a critical evaluation of the quality and relevance of the data reviewed. In this regard, the SAB recommends that the draft assessment be explicit as to whether the available evidence does or does not support each of the discussed systemic effects as a potential human hazard, and the rationale for reaching that conclusion. In this regard, the <u>meaning</u> of the statement (line 11, p. 1-61 and lines 13 & 14, p. 1-70) "at this time no conclusions are drawn regarding "xxxxx" [*viz.* liver effects or other non cancer effects] as human hazards of RDX exposure", is <u>not clear.</u> Does the draft assessment mean to state that existing data are inadequate to establish that RDX can cause a particular adverse effect in humans, or do that existing data are inadequate quantitatively to serve as a point of departure for a risk assessment? It is clear, for example, that very high unspecified doses of RDX cause modest, reversible increases in liver-specific serum enzyme activities in humans. High RDX doses cause modest increases in serum enzyme activities and hepatomegaly in dogs. RDX does not appear to enhance serum enzymes in rats, but does produce increased liver weight. However, the increases in relative liver weights are not consistently seen from one study to another. In light of this example, RDX clearly does cause modest hepatic effects in humans and animals, though there are no dose-response data available to calculate a RfD. Simply stating that "no conclusions are drawn regarding liver effects as a human hazard of RDX exposure" leaves the reader uncertain as to <u>what decision</u> EPA has made and why.

The description of Liver Effects in Section 1.2.4 is well written and comprehensive. The authors have done an excellent job grouping studies and providing detailed accounts. Conclusions about consistency of inter- and intra-species findings of different durations are quite reasonable. Integration of the liver effects on the top of the page 1-61 should lead to a more <u>specific/definitive</u> conclusion, as addressed above, than as stated in lines 10 and 11.

It is recommended that the overviews of ocular, cardiovascular (CV), musculoskeletal (MS), immune, gastrointestinal (GI) and hematological effects be moved from Section C.3.2 of Appendix C to a new subsection 1.2.5 (Other Noncancer Effects), rather than be part of an Appendix that readers may not readily access. As such, section 1.2.5 (Carcinogenicity) would become section 1.2.6.

The accounts of ocular, cardiovascular system, musculoskeletal system, immune system, gastrointestinal system, and hematological system effects of RDX, like those for the liver, are generally detailed, accurate and comprehensive in their coverage of each organ system. It is laudable that each account except for the musculoskeletal system, is concluded by a definitive, well-supported <u>summary</u> statement. The following additional information may be helpful in developing conclusions and related rationale.

It is stated in lines 24-26 of page C-44 that muscle injury was indicated by elevated levels of AST or myoglobinuria. However, some other enzymes measured in serum are more specific for muscle damage. Kucukardali et al. (2003), for example, reported transient increases in several serum enzymes in 4 of 5 patients experiencing RDX-induced seizures. One of the enzymes, creatine phosphokinase (CPK), is primarily indicative of muscle damage. Testud et al. (2006) measured elevated CPK and myoglobin levels in an Octogen-poisoned patient. The clinicians attributed these findings to muscle damage secondary to seizures.

It is concluded in lines 5 and 6 of page C-46 that histopathological changes have generally not been reported in RDX dietary studies. Kucukardali et al. (2003) did observe gastroduodenitis by endoscopy in 3 of 5 human poisoning victims who ingested enough RDX to cause protracted seizures. Severe irritation of the GI mucosa by direct contact with RDX would account for the nausea and vomiting commonly experienced by humans who ingest high doses of the chemical.

With respect to effects of RDX on the immune system, the empirical data have been summarized adequately in Appendix C.3.2. Based on the available animal studies, consistent dose-related immune system effects from oral exposure to RDX were not observed. However, it should be noted that none of these studies, including that of Crouse et al. (2006), included sensitive immune <u>function</u> evaluations. Crouse et al. (2006) was specifically designed to evaluate immunotoxicity in rats, but included only less sensitive structural evaluations of the immune system, such as populations of red and white blood cells, proportion of cell surface markers, cellularity in proportion to organ weight, B and T cells in the spleen, and CD4/CD8 antigens of maturing lymphocytes in the thymus). As observed by USEPA (1998), WHO (2012), and others, evaluation of such structural parameters in the absence of more sensitive functional testing is unlikely to detect immunosuppression, unintended immune stimulation, autoimmunity, or dysregulated inflammation.

Importantly, neuroinflammation has emerged as a key characteristic of most neurological conditions, including seizure and epilepsy, as recently reviewed by Dey et al. (2016) and Eyo et al. (2017). In particular, RDX-induced seizures may trigger acute immune and inflammatory responses within the brain, while chronic neuroinflammation may result from recurrent seizures. Neuroinflammation, in turn, has a proconvulsant effect by lowering the seizure threshold, influencing seizure severity and recurrence. This context is relevant to the interpretation of studies in which RDX exposures provoked convulsions. It is less clear what relationship, if any, there may be between less severe manifestations of RDX neurotoxicity and neuroinflammatory or other chronic immune system responses.

Not addressed in the draft assessment were the dose-related effects on body weights and/or body weight gains, although this was identified as a potential adverse effect of RDX elsewhere (e.g., Sweeney et al. 2012a,b; EPA, 2012b). Dose-related decreases in body weight gain were frequently observed in repeated dose studies with RDX, and should also be considered and discussed as a potential noncancer effect. Reduction in body weight is a common manifestation of adverse effects of chemicals, reflecting generalized systemic toxicity. This parameter has been utilized in numerous IRIS assessments for the derivation of reference values. The RDX literature should be screened to identify subchronic or chronic animal studies in which dose-dependent decreases in body weight/body weight gain are reported. Dose-related body weight effects should be discussed in the draft assessment, including their suitability to carry forward from hazard identification to the dose-response analysis.

Recommendations

- For each of the other noncancer hazards discussed in the draft assessment, add a conclusion regarding whether the available studies do or do not support a conclusion that the identified toxicity is a potential human hazard. Include an explanation of the rationale for reaching the conclusion, taking into consideration the additional information pertaining to liver effects, the muscle injury, immune system, neuroinflammation and gastrointestinal effects, as detailed above by the SAB.
- Include as a potential noncancer hazard the available subchronic and chronic data on body weight/body weight gain, and whether the studies do or do not support a conclusion that body weight effects represent a potential systemic human hazard. Discuss the rationale for the conclusion and explain why body weight effects are or are not carried forward to the dose-response analysis.
- Move the overviews of ocular, cardiovascular, musculoskeletal, immune, gastrointestinal and hematological effects from Section C.3.2 of the Appendix C to Section 1.2 of the main body of the draft assessment. These overviews should be placed in subsection 1.2.5 (Other Noncancer Effects) rather than be part of an Appendix. Section 1.2.5 (Carcinogenicity) would become section 1.2.6.

3.3.5. Cancer

3.3.5.1. Cancer Hazard

Charge Question 3e(i). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

The SAB concurs with the EPA that "*suggestive evidence of carcinogenic potential*" is the most appropriate cancer hazard descriptor for RDX and that this descriptor applies to all routes of human exposure.

In the draft assessment the EPA considered two potential hazard descriptors under the EPA's *Guidelines for Carcinogenic Risk Assessment* (USEPA, 2005): "*likely to be carcinogenic to humans*" and "*suggestive evidence of carcinogenic potential*," with the latter indicative of a lesser weight of evidence. According to the guidelines, the *suggestive evidence of carcinogenic potential* descriptor is "appropriate when the weight of evidence is suggestive of carcinogenicity, a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion." A *likely to be carcinogenic in humans* descriptor is appropriate when "the weight of evidence is adequate to demonstrate carcinogenic potential to humans" but is not strong enough to justify the highest weight of evidence descriptor *carcinogenic in humans*.

The draft assessment noted that RDX tested positive in more than one species, sex, and strain in animal studies and that this evidence was consistent with a "*likely to be carcinogenic in humans"* descriptor, as provided in the EPA guidelines (USEPA, 2005), and suggested that more than one descriptor might apply to RDX. However, the draft assessment also noted that the evidence of carcinogenicity outside the

B6C3F1 mouse was not robust, and this factor was decisive in choosing a hazard descriptor, which was "*suggestive evidence of carcinogenic potential"*.

In considering the most appropriate cancer hazard descriptor for RDX, the SAB evaluated the strength of evidence for positive cancer findings. The SAB agreed with the EPA that the relevant observations are the liver tumors that were observed in female B6C3F1 mice and male F344 rats and lung tumors that were observed in female B6C3F1 mice in two-year dietary bioassays (Lish et al. 1984; Levine et al. 1983) and identified a number of limitations that raised concerns. The findings of the SAB are as follows:

1) *Mortality in the high dose groups*. The high dose in the Lish et al. dietary study in mice was initially 175 mg/kg; however, the dose of RDX was reduced at week 11 of the study to 100 mg/kg diet, due to acute toxicity and high early mortality (30 of 65 males and 36 of 65 females) in the high dose animals. The acute toxicity and early mortality reduced the effective number of animals after 11 weeks on the study to 35 male mice and 29 female mice. Twenty-two male and 25 female mice in the high dose group survived to the scheduled sacrifice at 24 months. Similarly, the high dose in the Levine et al. dietary study in rats was 40 mg/kg, and mortality was high throughout the study period of 24 months. Unlike the mouse study, mortality occurred gradually over the entire period of the rat study, with mortality in most rats occurring after 6 months. Male rats were particularly affected by RDX toxicity, and histopathological evaluations indicated that the high mortality was likely due to renal disease. Four of 55 males and 28 of 55 females in the 40 mg/kg dietary exposure survived to scheduled sacrifice. The *Guidelines for Carcinogen Risk Assessment* states that "If toxicity or mortality is excessive at the high dose, interpretation [of cancer] depends on whether or not tumors are found. … Studies that show tumors at lower doses, even though the high dose is excessive and may be discounted, should be evaluated on their own merits."

2) *Liver tumors in rats*. A positive finding of cancer hazard in two species is based on the liver tumor response in male F344 rats to RDX in addition to the liver and lung tumors observed in female RDX-exposed mice. The liver tumor response of males to RDX in the Levine et al. (1983) rat study was only significant in a trend test, if the incidence of hepatocellular carcinomas in males of the high dose group (40 mg/kg-day) was included. When this group was omitted from the analysis because of its high mortality, the trend was not significant nor was a pair-wise comparison of the high dose group incidence to that of control males. There was no dose-related trend for the incidence of adenomas or the combination of adenomas and carcinomas. Although the incidence of benign liver tumors in control males may have been on the high end of the range for historical controls (Haseman et al. 1985), the evidence for an association of RDX exposure with increased liver tumors in this rat study is weak and does not support a carcinogenic effect of RDX.

3) *Lung tumors in mice*. The increased incidences of tumors in liver and lung observed in female $B6C3F_1$ mice would support a positive finding of cancer hazard at two sites. However, the lung tumor response to RDX in the mouse study of Lish et al. (1984) showed a significant trend for an increase only when the incidence of lung tumors (adenomas and carcinomas combined) in females of the high dose group (175/100 mg/kg-day) were included in the trend test. The trend and pairwise comparison tests were not significant if the high dose group was excluded from the analysis. A positive trend for the incidence of pulmonary carcinomas (not adenomas) was observed in both

sexes of mice, but only when the high dose groups were included. The incidence of these tumors was quite close to that observed in historical controls (Haseman et al. 1985). Thus, the evidence for an association of RDX exposure with increased lung tumors in this mouse study is weak and solely driven by the findings in the high dose group that suffered from high early mortality.

4) *Liver tumors in mice*. A positive finding of cancer hazard in both sexes of one species is based on the liver tumor response in male and female $B6C3F_1$ mice to RDX, but the SAB identified several concerns regarding liver tumors in mice.

- Although there were suggestive increases in liver tumors in male mice, none of the increases appear to be statistically significant using either a trend test or pairwise comparison tests. Of note, the incidences of these tumor types are quite variable in mice and the observed increases are within the range of incidences observed in historical controls (Haseman et al. 1985). Of further note is that the incidence of combined adenoma and carcinoma liver tumors observed in the high dose group is near the high end of the historical control range and that increases in tumors at other sites were not observed in male mice.
- The liver tumor findings were more robust in female mice, but there were also concerns with these observations due to the unusually low incidence of hepatocellular tumors in female control mice. None of the concurrent female mice controls had hepatocellular carcinomas (0.0%) and 1 of 65 had a liver adenoma (1.5%), while historical incidence control data published by the NTP were 8.0% for the combined hepatocellular carcinoma and/or adenoma, indicating that the observed 1.5% incidence was notably at the low end of the range of incidences found in historical controls.
- In a reevaluation of hepatocellular neoplasms in female mice by a Pathology Working Group (PWG), the original histological sections from female mice were retrieved and a second examination was performed by a pathologist (Parker et al. 2006). It was noted that a reevaluation of neoplasm sections from just one sex is unusual; sections from both male and female animals would be reevaluated typically to ensure that findings in both sexes were reliable. Members of the PWG then reexamined all hepatocellular neoplasm sections from female mice and cited factors that reduced their confidence in a positive interpretation of the study. These included variations in the number of liver sections per mouse, the absence of precursor lesions, such as foci of cellular alteration, and, most importantly, the low incidence of hepatocellular neoplasms (1.5%) in the control females. A discrepancy in the number of mice examined by Lish et al. (1984) and by Parker et al. (2006), while not major, further undermined the confidence in the quality of the data.

5) Non-neoplastic histopathological changes in the liver were absent in the majority of subchronic studies available in the literature, and pre-neoplastic lesions were absent in the livers of mice and rats at interim sacrifices conducted at 6 and 12 months in the two-year bioassays by Lish et al. (1984) and Levine et al. (1983). The finding that non-neoplastic changes in livers were not associated with RDX exposure in the majority of animal studies suggested that intrinsic factors may be involved in the observed tumor findings, especially in light of the fact that the mode of action of RDX carcinogenicity cannot be determined based on the current understanding of RDX metabolism (see below). It is acknowledged that the absence of hepatic precursor lesions does not, in itself, negate the possibility that RDX could have caused the increases in liver neoplasms, but nevertheless is a weight of evidence factor to consider.

6) *The lack of pathology peer-review and available data to support mortality-based statistics for neoplasms in the two-year bioassays by Lish et al. and Levine et al*. Carcinogenicity findings in

well-conducted experimental animal studies are regarded as evidence of potential cancer risk to humans by national and international health agencies. In order for experimental animal studies to serve as reliable sources of data for the evaluation of the carcinogenic potential of environmental agents, certain criteria must be met (Melnick et al. 2008). These include: a) animal models that are sensitive to the end points under investigation; b) detailed characterization of the agent and the administered doses; c) challenging doses and durations of exposure (approximately 2 years for rats and mice); d) sufficient numbers of animals per dose group to be capable of detecting a true effect; e) multiple dose groups to allow characterization of the dose-response relationships; f) complete and peer-reviewed histopathologic evaluations; and g) pairwise comparisons and analyses of trends based on survival-adjusted tumor incidence (Melnick et al. 2008). The Lish et al. and Levine et al. studies met criteria a – e; however, complete and peer-reviewed histopathologic evaluations and pairwise comparisons and analyses of trends based on survival-adjusted tumor incidence were not conducted and the available data did not allow EPA to perform these analyses. Additionally, necropsy and histological processing records were not available to link trace gross lesions observed at necropsy or the number of gross lesions with histological sections that were evaluated for each animal.

7) <u>*Limited evidence to support a mode of action for RDX carcinogenicity.*</u> Data are not available in the literature to support the metabolism of RDX by human liver or lung enzymes or by human microflora to form genotoxic agents. One rodent study shows reductive transformation of RDX to N-nitroso compounds (Pan et al. 2007b). It is unclear if this transformation occurred via microflora, non-enzymatic processes, or by rodent metabolic enzymes. Bhushan et al. (2003) reported that rabbit cytochrome P450 2B4 converts RDX to 4-nitro-2,4-diazbutanal *in vitro*. This compound and 4-nitro-2,4-diaza-butanamide were identified as minor end product metabolites in urine of Yucatan miniature pigs (Major et al. 2003). However, the genotoxic potential of these compounds has not been determined in mutagenesis assays. Numerous studies have shown that RDX yields negative test results with the Ames *Salmonella typhimurium* assay in a variety of bacterial strains (Cholakis et al. 1980; George et al. 2001; Tan et al. 1992) and is not cytotoxic or mutagenic in the in vitro mouse lymphoma test or in vivo by the mouse bone marrow micronucleus test (Reddy et al. 2005). RDX was reported by one group to be weakly mutagenic in one strain of Salmonella typhimurium using S9 activation (Pan et al. 2007a) and showed some evidence of mutagenic activity in *Vibrio fischeri* using the Mutatox assay (Arfsten et al. 1994). In vitro biotransformation studies on RDX suggest that RDX can be metabolized by anaerobic bacteria in soils to form N-nitroso derivatives, which may be further transformed into genotoxic alkylhydrazines; however, the extent and persistence of these products are not known (McCormick et al. 1981). Whether rodent or human microflora can produce these alkylhydrazine metabolites *in vivo* remains unknown at the present time. Other modes of action of RDX, such as oxidative stress mechanisms in carcinogenesis, have not been investigated.

Based on the guidance provided in the EPA's *Guidelines for Carcinogenic Risk Assessment* (USEPA, 2005) and points 2-4 above, the SAB considers that the evidence for a positive tumor response to RDX in two species, two sexes, or two sites required by EPA for a "*likely to be carcinogenic in humans"* descriptor is weak or absent. Given the limitations and nature of the carcinogenicity data available, the SAB concluded that the descriptor, "*suggestive evidence of carcinogenic potential"*, is appropriate. As noted in the draft assessment and in the discussion above, oral exposure to RDX has been observed to result in tumors in liver, which is beyond the point of initial contact. This is indicative of carcinogenic effects that are systemic rather than confined to the portal of entry to the body, and thus carcinogenic potential is independent of the route of exposure. Therefore, the SAB agrees with EPA that this descriptor applies to all routes of exposure.

Recommendations

- The draft assessment should expand on the limitations of the Lish et al. (1984) and Levine et al. (1983) animal studies. For example, clarification could be provided that the absence of hepatic precursor lesions in the female mice of the Lish et al. (1984) study does not, in itself, negate the possibility that RDX could have caused the increases in liver neoplasms; and a more complete description could be included to discuss the differences in mortality time course between mice in the Lish et al (1984) study and rats in the Levine et al. (1983) study administered the high dose level of RDX in the diet and the potential impact of these differences on the interpretation of the hepatic and pulmonary neoplasms in female mice.
- Strengthen and make more specific the description of the justification for selection of the "*suggestive evidence of carcinogenic potential"* descriptor rather than the "*likely to be carcinogenic to humans"* descriptor.

3.3.5.2. Cancer-specific Toxicity Values.

Charge Question 3e(ii). As noted in EPA's 2005 Guidelines for Carcinogen Risk Assessment, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

The SAB finds that the draft assessment adequately explains the rationale for a quantitative analysis of RDX cancer assessment and that the selection of the Lish *et al*. (1984) study for this purpose is supported scientifically and clearly described.

Despite concerns associated with interpretation of the data discussed in response to Charge Question 3e(i), the Lish et al. (1984) study was a well-conducted two-year bioassay that included a large number of animals tested at multiple dose levels, and increased incidences of neoplasms occurred in exposed female mice. This study is suitable and appropriate for dose-response assessment, consistent with the EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005).

3.3.5.3. Point of Departure for Cancer Endpoints.

Charge Question 3e(iii). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

The SAB finds that the calculations in the draft assessment of the PODs and oral slope factors for cancer endpoints are not clearly described, and the SAB has questions about whether these are scientifically supported. A number of concerns were expressed with the data used to compute the cancer POD, the rationale for restricting modeling to the multistage model, and the conditions under which the MS-COMBO methodology provides a valid POD and cancer slope factor estimate.

The draft assessment discusses two modes of action for cancer, genotoxicity and cell proliferation, but the available data are too limited to support use of a mechanism-based model. Without a clear mode of action, the linear low-dose extrapolation method recommended in the EPA 2005 Cancer Guidelines is used in the draft assessment. The SAB agrees with this choice.

The SAB has concerns with the low incidence of liver tumors (hepatocellular adenomas and carcinoma) in female mice and its impact on dose response modeling. As indicated in Section 1.2.5, the 1.5% incidence of liver tumors in the control B6C3F1 mice is unusually low. This was reported by the study authors as significantly lower than reported for historical controls, and lower than the incidences reported for this mouse strain by the National Toxicology Program (NTP) (mean 8%, range 0-20%). This unusually low control incidence could significantly influence the estimate of the POD. The SAB recommends that for liver cancer, additional BMD modeling (i.e. a sensitivity analysis) should be performed to examine and illustrate the impact of low concurrent controls on model choice and POD estimate.

Concerns that inclusion of the highest dose in the dose-response model for liver tumors for the B6C3F1 mouse study may impact the POD estimate and estimated cancer slope factor were discussed. The previous RDX risk assessment excluded the high dose used in this study in deriving the POD and cancer slope factor. A change in the highest dose at week 11 due to high mortality was reported, and mice that died prior to week 11 were excluded from the analysis. This results in a reduced sample size for the highest dose group from 65 to 31 animals and subsequent increased uncertainty in the response to this dose. While survival times of mice in the highest dose group were not significantly different from other dose groups, high mortality in the early weeks may mean that remaining survivors had other differences that potentially resulted in higher resistance to cancer. Excluding the highest dose group results in a fitted multistage model form that is almost linear. Using this fitted model produces an estimated POD that is much less than that estimated with the highest dose group included. This alternate POD in turn produces an unrealistically high cancer slope estimate (see Figure D-15). The SAB had no recommendation on how to deal with this issue other than to include/exclude the highest dose in the sensitivity analysis for examining concurrent controls.

The draft assessment relies on the multistage model to describe dose response relationships and subsequently to estimate the POD and cancer slope factor. As discussed in the BMDS guidance documents, the IRIS program prefers using a multistage model when no biological basis for model form is available because it considers this class of models as sufficiently flexible to address the typical dose response patterns of cancer bioassay data, and its use encourages comparability across IRIS assessments. The SAB did not find this rationale for using the multistage model discussed in the draft assessment, and this omission led the SAB to question other aspects of the dose response modeling as well as the use of the MS-COMBO package. Further, the SAB concluded that more discussion around why the multistage model is used and how EPA typically assesses the multistage model fit would greatly improve understanding and reduce confusion. While a discussion of the benefits and weaknesses of the multistage modeling approach is also in the BMDS guidance document, a summary of these should also be provided. While understanding the preference of the IRIS program for the multistage model form, the SAB recommends that at a minimum the draft assessment should discuss the adequacy of the fit of the multistage model to available data. This discussion could be further supported by exploring and reporting fits to other standard BMD model forms.

The SAB expressed concerns that the *assumption of independence of tumor incidence* that is required by the MS-COMBO methodology used in estimating the POD is not clearly described. In addition, there is no discussion around the validity of this assumption for the available RDX data. However, these data are available in the pathology report in Lish et al. (1984), and joint occurrence of liver and lung tumors was found in only one animal in the 175/100 mg/kg-day dose group and one animal in the 35 mg/kg-day dose group, demonstrating that the assumption of independence of tumor incidence holds. Finally, the SAB could not determine whether the MS-COMBO methodology requires that the dose- response for each tumor be adequately described by a multistage model, and whether the tumors being combined must be adequately fit by the same multistage model form. The SAB recommends that a better and more detailed description of the MS-COMBO methodology be provided in the draft assessment and that this description clarify the points raised above. In particular, a test that better describes the independence assumption and the impact of violations of this assumption on the estimated POD should be included.

3.4. Dose-Response Analysis

3.4.1. Oral Reference Dose for Effects other than Cancer.

Charge Question 4a. The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous system effects as described in the Crouse et al. (2006) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

EPA has done a reasonably good job in terms of clearly describing the process and choices made to arrive at this oral RfD. The SAB finds that the overall RfD for RDX should be based on nervous system effects. Neurotoxicity was observed in multiple animal studies and in exposed humans, and included hyperactivity, hyperirritability, tremors and convulsions. Mechanistic data supports the neurotoxic effects of RDX, namely binding to the $GABA_AR$ and antagonizing GABA-mediated post-synaptic inhibition. EPA also provides an RfD based on suppurative prostatitis and another based on testicular degeneration. The SAB finds that suppurative prostatitis, which EPA describes as a surrogate for the entirety of effects of RDX on the kidney and genitourinary system, is not the most appropriate toxicological endpoint for the overall RfD. There is no known mechanistic link between suppurative prostatitis and renal papillary necrosis or adverse effects on renal function. Thus, suppurative prostatitis does not provide any indication of adverse effects in the kidneys. The SAB also found that testicular degeneration was not an appropriate endpoint to serve as a basis for the overall RfD. Testicular degeneration was reported at terminal sacrifice (24 months) in one 2-year dietary study in mice (Lish et al. 1984). The SAB noted that testicular histopathology should have been seen at earlier time points (e.g., the 6 months and 12 months interim sacrifices) in Lish et al. (1984), as these exposure durations were several times longer than the 1.4-month duration of spermatogenesis in mice. Germ cell degeneration was also observed in a 2-year dietary study in rats (Levine et al. 1983) but only at the 12-month interim sacrifice, and not at the 6-month interim or 24-month terminal sacrifice. Furthermore, testicular degeneration was not observed in the majority of the dietary and gavage studies in rodents (5 of 7 showed no effect).

Although the SAB agrees that neurotoxicity should be the basis for an overall RfD for RDX, the SAB finds that the scientific support for the proposed oral RfD is weak. In particular, the choice of Crouse et al. (2006) as the basis for the RfD does not address the confirmed convulsion in an exposed pregnant female at 2 mg/kg-day in Cholakis et al. (1980). This occurred at a dose below the LOAEL of 8 mg/kg-day in the Crouse et al. (2006) study.

EPA chose the Crouse study over the Cholakis study for several stated reasons: lack of specific monitoring for neurological effects (e.g., convulsions) in Cholakis study; a higher purity test material in Crouse et al. (2006); fewer dose groups and wider spacing of dose groups in Cholakis et al. (1980) compared to Crouse et al. (2006); and longer exposure duration in Crouse et al. (90 d) compared to Cholakis et al (14 d). While acknowledging that the Crouse study was a better designed study to detect neurological effects, the SAB concludes that the Cholakis et al. (1980) study is a better choice for derivation of an RfD based on convulsions. Notwithstanding that the monitoring for neurological effects in Cholakis et al. (1980) was incomplete, the observation of a (single) rat with convulsions at 2 mg/kg-day was a valid observation and the fact that it was noted despite incomplete observation suggests the possibility that this dose may overestimate the true LOAEL. The SAB acknowledges that the greater purity of the test material in Crouse et al. (2006) is a matter for some uncertainty in the choice of the key study. However, the SAB does not believe that the difference in purity is sufficiently great that this factor alone should determine the choice of study. Rather, it should be viewed in the context of the overall uncertainty of study choice. The more informative dose spacing in Crouse et al. (2006) can, potentially, allow for less uncertainty in dose-response modeling. However, the SAB believes that the empirical observation of convulsion at the lower dose in Cholakis et al. (1980) is more informative than the model-based prediction below the LOAEL in Crouse et al. (2006). Finally, the exposure duration was considerably longer in Crouse et al. (2006), and the potential advantage of longer exposure duration is that it may show effects at lower doses than in other studies of shorter duration even with the same dose range. Since the 14-day LOAEL from Cholakis et al. is lower than the 90-day LOAEL from Crouse et al., it does not appear that the longer exposure duration in Crouse et al. provides a practical advantage in this case.

Given these considerations, the SAB believes that an oral RfD based on convulsions should specifically and quantitatively take the LOAEL from Cholakis et al. (1980) into account. The SAB considered three possible options for this:

1. <u>Conduct a benchmark dose analysis on the convulsion data from Cholakis et al. (1980).</u>

EPA provided information that the incidence of convulsions at the high dose in the Cholakis et al. study was combined with the incidence of other neurologic effects. The response at this dose is, therefore, not appropriate for inclusion in benchmark dose modeling. However, elimination of the high dose from the Cholakis et al. dose-response data leaves only one effective dose and this does not provide an adequate basis for dose-response modeling. The SAB, therefore, rejected this option.

2. <u>Combine the dose-response data from Cholakis et al. (1980) and Crouse et al. (2006)</u>

There were several impediments to this approach. The two studies had different exposure durations (Cholakis et al. (1980),14-day; Crouse et al. (2006), 90-day). Sex and pregnancy status differed between the studies (Crouse et al., males and females; Cholakis et al., pregnant females only). Therefore, the SAB also rejected this option.

3. Use the NOAEL (0.2 mg/kg/d) from Cholakis et al. (1980) as the POD

The SAB identified several advantages to using this approach. As noted above, with the elimination of the high dose from Cholakis et al. (due to inclusion of non-convulsive effects), there is no basis for benchmark dose modeling anyway, and a NOAEL is an appropriate basis for a POD. This approach eliminates issues around the choice of a BMR from Crouse (see response to charge question 3a(iii)), and addresses the SAB's concern with the existence of a lower LOAEL from Cholakis et al. compared to Crouse et al.

Table 4 below provides a comparison between EPA's proposed value (first row entry) based on the 1% BMR from Crouse et al, (2006) with alternate RfDs. If the same UFs (composite UF of 100) are applied to the PBPK-adjusted NOAEL POD from the Cholakis study as EPA applied to the PBPK-adjusted BMDL POD from the Crouse study, the RfD based on Cholakis et al. (1980) would be 1 x 10^{-3} mg/kg/day. Applying the SAB recommended composite UF of 300 to the PBPK-adjusted NOAEL POD from Cholakis et al (1980) results in an RfD of 3 x 10^{-4} mg/kg-day. Applying the SAB-recommended composite UF of 300 to the 1% BMR from Crouse et al. would result in an RfD of 1 x 10^{-3} mg/kg-day. If, however, the RfD from Crouse et al. were calculated based on a BMR of 5% as recommended by the SAB (see response to charge question 3a(iii)), applying the recommended composite UF of 300, the RfD would be 4 x 10^{-3} mg/kg-day. These RfDs can be compared to the EPA's proposed RfD of 3 x 10^{-3} mg/kg-day from Crouse et al., based on a BMR of 1% and a composite UF of 100.

Table 4. Comparison of Derived Candidate RfDs

Reference	**POD (mg/kg-day)**	**POD Type**	**POD_{HED}[a]**	**Composite UF**	**RfD value (mg/kg-day)**
Crouse et al, 2006	0.57	BMDL01	0.28	100	0.003
Cholakis et al. (1980)	0.2	NOAEL	0.097	100	0.001
Cholakis et al. (1980)	0.2	NOAEL	0.097	300	0.0003
Crouse et al. (2006)	0.569	$BMDL_{01}$	0.28	300	0.001
Crouse et al. (2006)	2.66[b]	$BMDL_{05}$	1.295	300	0.004

a POD_{HED} is calculated from POD x PBPK derived adjustment factor of 0.487
b $BMDL_{05}$ is from response to charge question 3a(iii)

Consideration of mortality

The SAB understands this portion of the charge question as asking whether an RfD based on convulsions (from either Crouse et al. (2006), or Cholakis et al. (1980)) is adequately protective against lethality. The SAB agrees that mortality and convulsions are linked. However, the SAB is not aware of any evidence for RDX or similar seizurogenic compounds where neurologic mortality occurs in the absence of convulsions. The overall RfD recommended by the SAB is based on a NOAEL for convulsions of 0.2 mg/kg-day. This can be compared to the NOAEL for convulsions of 10 mg/kg-day with no mortality in the monkey study of Martin and Hart (1974). The SAB believes that this provides some confidence that an RfD based on the NOAEL from Cholakis et al. provides a margin of safety with respect to neurologic-based lethality. However, the SAB acknowledges that the Martin and Hart study had a small sample size. The SAB, therefore, endorses increasing the database uncertainty factor to provide an appropriate margin of safety between convulsive and lethal neurologic effects (as well as accounting for data gaps in developmental neurotoxicity and lack of incidence data for less severe neurotoxic effects).

3.4.2. Inhalation Reference Concentration for Effects other than Cancer

Charge Question 4b. The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

There are no toxicokinetic data from inhalation exposure of laboratory animals or humans to RDX. There are epidemiological studies of persons exposed occupationally to RDX (Ma and Li, 1993; Hathaway and Buck, 1977), but no information was provided on exposure levels. These workers were likely exposed dermally and by inhalation. In light of the lack of toxicokinetic data and exposure levels, inhalation reference concentration cannot be derived.

3.4.3. Oral Slope Factor for Cancer

Charge question 4c. The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

The SAB finds that in the draft assessment the calculation of an oral slope factor (OSF) for cancer endpoints is not clearly described and, borrowing from the discussion in question 3e(iii), the SAB has questions about whether the OSF is scientifically supported. The SAB makes multiple suggestions for how the discussion on the derivation of the OSF can be improved.

The OSF is estimated as the plausible upper-bound (95% upper CI) for the true slope, or risk per unit dose, from which the probability that an individual will develop cancer if exposed to an agent for a lifetime of 70 years can be computed. In practice, and as done in the draft assessment, the OSF for the cancer endpoint is obtained as the slope of the line from a point of departure, in this case the $BMDL_{10\%}$, to the estimated control response at a dose of zero. Consequently, any changes to the derivation of the POD will be reflected in the estimate of the OSF. In its response to question 3e(iii), the SAB identifies a number of concerns with the data used to compute the cancer POD, and offers recommendations for improving the calculation of the POD. These recommendations will change the estimated POD and thus the OSF.

The draft assessment proposes combining tumors from different sites in determining an overall cancer risk. The SAB finds that this is both logically and toxicologically sound. While not discussed in either the draft assessment or the draft supplemental material, the independence of tumor location is a key assumption for acceptable use of the MS-COMBO model. The SAB was able, using original study data, to determine that there is little biological or statistical support that the two tumors used in the MS-COMBO analysis have dependent tumor incidence, hence the assumption of independence is concluded, and the MS-COMBO approach is valid. However, as discussed in the SAB's response to question 3e(iii), a number of issues around the use of the MS-COMBO model remain to be clarified.

The SAB expressed concern that the near linearity of the fitted multi-stage dose-response models (see Table 2-7 in the draft assessment identifying all selected models as Multistage 1°) results in a relatively poor fit (model estimates) at the highest doses. The two fitted models used (see Figures D-12 and D-14 in the RDX draft supplement document) have $BMDL_{10\%}$ estimates that are larger than the two lowest non-zero doses used. From the Cancer Guidelines (page 3-16) "*If the POD is above some data points, it can fail to reflect the shape of the dose-response curve at the lowest doses and can introduce bias into subsequent extrapolations*." This seems to be what is happening with the RDX-induced adenomas and carcinomas data, and the issues with the $BMDL_{10\%}$ seem to arise primarily because the fitted multistage models (with parameter constraints invoked) lack sufficient curvature. Larger than expected $BMDL_{10\%}$ values (the PODs) result in lower estimated OSFs. The SAB conjectures that using a model form that allows more curvature could provide a better fit at the mid-range and higher doses and improve the quality of fit. As mentioned in the response to question 3e(iii), the SAB acknowledges that EPA standard practice is to use the multistage model for benchmark dose modeling of cancer dose-response when there is no biological basis for choosing another model. In this case though, the relatively poor fit of the multistage model to the hepatocellular and alveolar/bronchiolar adenomas and carcinomas data produces an estimate of the POD with poor properties. The SAB recommends that at a minimum the draft assessment discuss the adequacy of the fit of the multistage model to available data**.** This discussion could be further supported by exploring and reporting fits to other standard BMD model forms – engaging in a curve-fitting exercise starting for example with the list in Table D-13 in the draft supplemental document. Although the multi-stage model does ensure positive slopes throughout, the BMDS software facilitates fitting other models that also adhere to this constraint.

The SAB expressed concern that the results for liver cancer in concurrent female control mice were very low (1.5 %) compared to available historical control rates (8%; range 0-20%) (page 1-62 of the draft assessment). This low rate influences the final model for liver, which in turn significantly impacts the estimate of the POD and hence the OSF. It is not clear how this issue impacts the POD estimate derived via the MS-COMBO analysis where liver cancer results are combined with those of lung cancer to produce the final POD used. The SAB recommends that EPA acknowledge the low concurrent control liver cancer response in female mice and discuss its impact on the level of confidence in the final MS-COMBO estimate of the proposed POD.

The SAB also noted, and the draft assessment confirms (Section 1.2.5, page 1-61) that at the highest dose level in the Lish et al. (1984) study for the first 11 weeks, the animals had an elevated mortality strongly suggesting that the maximum tolerated dose had been greatly exceeded. At 11 weeks, the researchers lowered the dose, and it was a duration-weighted average dose level that was used as the highest dose in the fitting of the multistage model (see Section D.2.2 (pages D-31 to D-33) of the RDX draft supplement document). The Cancer Guidelines (page A-4) discuss this situation and offer that the decision to use or not use data from doses that exceed the MTD is "*a matter of expert judgement*". The SAB has concerns that including this dose significantly impacts the final estimated POD. The SAB recommends that additional insight be sought by fitting the multistage model and estimating the POD after exclusion of this dose level, comparing the POD generated from both models, and discussing why the estimate that is based on the use of the highest dose data is preferred. Following this analysis through to the MS-COMBO results also seems reasonable. This comparison and subsequent discussion is supported by the fact that the current IRIS entry for RDX of 0.11 per mg/kg-day was determined using the liver tumor data (Lish et al. 1984) with the highest dose values excluded.

3.4.4. Inhalation Unit Risk for Cancer

Charge Question 4d. The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

There are no toxicokinetic data from inhalation studies of RDX in laboratory animals or humans, no carcinogenicity bioassays of RDX, nor data on cancer incidence in humans. Therefore, an inhalation unit risk for cancer cannot be derived.

3.5. Executive Summary.

Charge Question 5. Does the executive summary clearly and adequately present the major conclusions of the assessment?

Generally, the SAB considered the Executive Summary to be well-written, succinct, and clear. As changes are made to the body of the draft assessment in response to the SAB's recommendations, the Executive Summary should be updated accordingly. In addition, the SAB had a number of content and editorial suggestions for improving the Executive Summary:

Characterization and description of urogenital system hazard and risk

- There is too much emphasis on the incidence and significance of the suppurative prostatitis. The other urogenital system effects are of more importance and should be described. The description of the urogenital effects in male rats should include specific mention of the renal effects (e.g., renal papillary necrosis and associated renal inflammation), not only the prostatic effects.

Because the observed suppurative prostatitis is part of a larger spectrum of prostatic inflammatory changes that are frequently found in aged F344 rats, the dose-response for this lesion found in male rats may in fact not reflect the overall incidence of all types of prostatitis combined in each dose group. The prostatic inflammation and renal/bladder effects may be inter-related, but this only occurred at the highest RDX dose tested, and there seems no basis for the assertion that suppurative prostatitis is a "surrogate marker" for renal/bladder effects. Therefore, an RfD based on suppurative prostatitis should be derived as a stand-alone endpoint, and a separate RfD should be derived for kidney and other urogenital system effects. Of note, this does not affect the overall oral reference dose, because that is based on the nervous system effects.

- In the section on "Suppurative prostatitis," the possibility of a bacterial infection is raised and its potential significance to RDX toxicity is briefly discussed. However, it should also be noted that this effect could also be secondary to inflammation without a bacterial infection.

- P xxiii line 7 – 9: The first sentence indicates human potential for kidney and urogenital toxicity, which is fine, but indicates this is "based on" increased relative kidney weights and histopathological changes. P 1-24 lines 24-30 indicates inconsistent findings in the subchronic studies and down-plays the organ weight findings in the chronic studies, so the executive summary is inconsistent with this.

Description of animal cancer bioassay results

Add the following to indicate some of the uncertainty or limitations in the animal cancer bioassay results.

- In the Summary, add "limited" to the following sentence as shown. Results from animal studies provide suggestive evidence of carcinogenic potential for RDX based on limited evidence of positive trends in liver and lung tumor incidence in experimental animals.

- In the body, add some clarifying or cautionary language on page xxv, line 26. In spite of limitations in the animal cancer studies, a quantitative estimate of carcinogenic risk.... or Cognizant of limitations in the animal cancer studies, a quantitative estimate of carcinogenic risk....

Other content clarification, missing information that should be included, and editorial comments

- P xxvii line 23 – 25: It is not clear, given the limited information on RDX exposures in the Preface or elsewhere in the draft assessment, that dietary exposure is "more representative of potential human exposures". It seems possible that human exposures could involve different or varied sources of RDX exposure (e.g., on swallowed dust particles, consumed soil, incorporated into plants) such that neither experimental exposures as diet nor as gavage would be obviously "representative". Thus, there would be uncertainty. Given this, please clarify the meaning of "more representative of potential human exposures," and be explicit regarding the uncertainty associated with identifying a representative experimental exposure.

- Include an explanation of the importance of RDX purity in published studies.

- Include the main criteria used for choosing the principal study.

- In the brief discussion of neurologic effects within the section, "Effects other than cancer observed during oral exposure," there is no mention of the lethality associated with convulsions. Include a discussion of the concordance in doses producing convulsions and doses at which death occurred in key animal studies.

- Provide a summary statement addressing the confidence (i.e., low, medium or high) in the RfD.

- On page xxiii, a paragraph break is needed after the sentence, "There is no known MOA for male reproductive effects of RDX exposure." The next sentence does not relate to the male reproductive effects but speaks to the evidence for effects in other organs/systems.

- On page xxv, the paragraphs titled, "Effects other than cancer observed following inhalation exposure" and "Inhalation reference concentration (RfC) for effects other than cancer," should be combined. There is no available literature to support the identification of hazards following inhalation and a reference concentration cannot be determined. This should be stated simply in a single paragraph.

REFERENCES

Angerhofer, R; Davis, G; Balezewski, L. (1986). Teratological assessment of Trinitro-RDX in rats. (75-5100573-86). Aberdeen Proving Ground, MD: U.S. Army Environmental Hygiene Agency.

Arfsten, DP; Davenport, R; Schaeffer, DJ. (1994). Reversion of bioluminescent bacteria (Mutatox) to their luminescent state upon exposure to organic compounds, munitions, and metal salts. *Biomed Environ Sci* **7,** 144-149.

Bach PH, Nguyen TK. (1998) Renal papillary necrosis--40 years on. *Toxicol Pathol*. 26(1):73-91.

Bannon, DI; Dillman, JF; Phillips, CS; Perkins, EL. (2009). Global gene expression in rat brain and liver after oral exposure to the explosive hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Chem Res Toxicol* 22: 620-625.

Beyer BK.; Chernoff, N; Danielsson, BR; Davis-Bruno, K; Harrouk, W; Hood, RD; Janer, G; Wandel Liminga U; Kim, JH; Rocc,a M; Rogers J; Scialli, AR. (2011) ILSI/HESI Maternal Toxicity Workshop Summary: Maternal Toxicity and Its Impact on Study Design and Data Interpretation. *Birth Defects Research* (Part B). 92:36–51.

Bhushan, B; Trott, S; Spain, JC; Halasz, A; Paquet, L; Hawari, I. (2003). Biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by a rabbit liver cytochrome P450: Insight into the mechanism of RDX biodegradation by Rhodococcus sp. Strain DN22. *Appl Environ Microbio* 69: 1347-1351. http://dx.doi.org/10.112B/AEM.69.3.1347-1351.2003

Brix, AE. (2002) Renal papillary necrosis. *Toxicol Pathol*.30(6):672-674.

Bronson, FH; Desjardins, C. (1977). Reproductive failure in aged CBF_1 male mice: Interrelationships between pituitary gonadotropic hormones, testicular function, and mating success. *Endocrinology* 101: 939-945.

Bruni, JF; Huang, HH; Marshall, S; Meites, J. (1977). Effects of single and multiple injections of synthetic GnRH on Serum LH, FSH and testosterone in young and old male rats. *Biol Reprod* 17: 309-312.

Cao, CI; Reddy, G; Bannon, DI; Hohnson,, MS. (2008). In vitro study of hexahydro-1,3,-5trinitro-1,3,5-triazine (RDX) metabolism in human liver. Aberdeen Proving Ground, ND: U.S. Army Center for Health Promotion and Preventive Medicine.

Carney, EW; Kimmel, CA, (2007) Interpretation of Skeletal Variations for Human Risk Assessment: Delayed Ossification and Wavy Ribs. *Birth Defects Research (Part B*)- *Developmental and Reproductive Toxicology* 80:473-496.

Chen, H; Hardy, MP; Zirkin BR. (2002). Age-related decreases in Leydig cell testosterone production are not restored by exposure to LH in vitro. *Endocrinology* 143: 1637–1642.

Cholakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III. (1980). Mammalian toxicological evaluation of RDX. (DAMD17-78-8027). Kansas City, MO: Midwest Research Institute.

Cohen, BJ; Anver, MR; Ringler, DH; Adelman, RC. (1978). Age-associated pathological changes in male rats. *Federation Proc* 37: 2848-2850.

Coleman, NV, Spain, JC, Duxbury, T. (2002). Evidence that RDX biodegradation by Rhodococcus strain DN22 is plasmid-borne and involves a cytochrome p-450. *J Appl Microbiol* 93: 463-472.

Creasy, D, Bube ,A, de Rij,k E, Kandori, H, Kuwahara, M, Masson, R, Nolte, T, Reams, R, Regan, K, Rehm, S, Rogerson, P, Whitney, K. (2012) Proliferative and nonproliferative lesions of the rat and mouse male reproductive system. *Toxicol Pathol*. 40(6 Suppl):40S-121S.

Creeley, CE. (2016). From drug-induced developmental neural apoptosis to pediatric anesthetic neurotoxicity – where are we now? *Brain Sciences* 6:32-44

Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI. (2006). Subchronic oral toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Dey, A.; Kang, X; Qiu, J; Du, Y, and Jiang, J (2016). Anti-Inflammatory Small Molecules To Treat Seizures and Epilepsy: From ench to Bedside. Trends in Pharmacological Sciences 37:(6) 463 - 484.

Eaton HL; DeLorme M; Craig AM. (2009). Metabolism of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Ovine Ruminal Microbes. *Microbial Ecology* 57:569-569.

Eyo, U. B., Murugan, M. and Wu, L.-J. (2017), Microglia–Neuron Communication in Epilepsy. *Glia* 65: 5–18. doi:10.1002/glia.23006

Fellows, RJ; Driver, CR; Cataldo, DA; Harvey, SD. (2006). Bioavailability of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) to the prairie vole (Microtus ochrogaster). *Environmental Toxicology and Chemistry* 25:1881-1886.

George, SE; Huggins-Clark, G; Brooks, LR. (2001). Use of a Salmonella microsuspension bioassay to detect the mutagenicity of munitions compounds at low concentrations. *Mutat Res* 490: 45-56.

Garcia-Reyero, N; Habib, T; Pirooznia, M; Gust, KA; Gong P; Warner, C; Wilbanks, M; Perkins, E. (2011). Conserved toxic responses across divergent phylogenetic lineages: a meta-analysis of the neurotoxic effects of RDX among multiple species using toxicogenomics. *Ecotoxicology* 20:580-594.

Gosden, RG; Richardson, DW; Brown, N; Davidson, DW (1982). Structure and gametogenic potential of seminiferous tubules in ageing mice. *J Reprod Fert* 64: 127-130.

Guo, L; Xu, H; Chen, Y; Chang, Y. (1985). Distribution and metabolism of tritium-labeled hexogen in white mice, *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi* 3: 335.330.

Gust, KA; Brasfield, SM; Stanley, JK; Wilbanks, MS; Chappell, P; Perkins, EJ; Lotufo, GR; Lance, RF. (2011). Genomic investigation of year-long and multigenerational exposures of fathead minnow to the munitiont compound RDX. *Environmental Toxicology and Chemistry* 30:1852-1864.

Halasz, A; Manno, D; Perreault, NN; Sabbadin, F; Bruce, NC; Hawari, J. (2012). Biodegradation of RDX Nitroso Products MNX and TNX by Cytochrome P450 XplA. *Environmental Science and Technology* 46:7245-7251.

Haseman, JK; Huff, JE; Rao, GN; Arnold, JE; Boorman, GA; McConnell, EE. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N X C3H/HeN)F1 (B6C3F1) mice. *J Natl Cancer Inst* 75: 975-984.

Hathway, JA; Buck, CR. (1977). Absence of health hazards associated with RDX manufacture and use. *J Occup Med* 19: 269-272.

Hess-Ruth, A; Crouse, L; Roszell, L. (2007). RDX pilot development neurotoxicity test in rats. (Toxicology Study No. 85-XC-064Y-07). Aberdeen Proving Grounds: U.S. Army Center for Health Promotion and Preventive Medicine.

Hollander, AI; Colbach,, EM. (1969). Composition C-4 induced seizures: A report of five cases. *Mil Med* 134: 1529-1530.

Jaligama, S; Kale, VM; Wilbanks, MS; Perkins, EJ; Meyer, SA. (2013). Delayed myelosuppression with acute exposure to hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and environmental degradation product hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX) in rats. *Toxicology and Applied Pharmacology* 266:443-451.

Jeilani, YA; Dunca,n KA; Newallo, DS; Thompson, AN Jr.; Bose, NK. (2015). Tandem mass spectrometry and density functional theory of RDX fragmentation pathways: Role of ion-molecule complexes in loss of NO3 and lack of molecular ion peak. *Rapid Communications in Mass Spectrometry* 29:802-810.

Johnson, L; Neaves, WB. (1983). Enhanced daily sperm production in the remaining testis of aged rats following hemicastration. *J Androl* 4:162-166.

Kim, JY; Liu, CY; Zhang, F; Duan, X; Wen, Z; Song, J; Feighery, E; Lu, B; Rujescu, D; St Clair D; Christian, K; Callicot, JH; Weinberger, DR; Song, H; Ming, Gl. (2012). Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. *Cell* 148:1051-1064.

Krishnan, K; Crouse, LCB; Bazar, MA; Major, MA; Reddy, G. (2009). Physiologically based pharmacokinetic modeling of cyclotrimethylenetrinitramine in male rats. *J Appl Toxicol* 29: 629-637. http://dx.doi.org/10.1002/jat.1455

Kucukardali, Y; Acar, HV; Ozkan, S; Nalbant, S; Yazgan, Y; Atasoyu, EM; Keskin, O; Naz, A; Akyatan, N; Gokben, M; Danaci, M. (2003). Accidental oral poisoning caused by RDX (cyclonite): A report of 5 cases. *J Intensive Care Med* 1B: 42-46. http://dx.doi.org/10.1177/0885066602239123

Levine, BS; Furedi, EM; Gordon,DE; Burns, JM; Lish, PM. (1981). Thirteen *week oral (diet) toxicity study of trinitrotoluene (TNT) and RDX in F344 rats. Fundam Appl Toxicol* 15: 3730380. http://dx.doi.org/10.1016/0272-0590(90)90062-0

Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM; (1983). Determination of the chronic mammalian toxicological effects of RDX (twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat): Final report—phase V, Chicago, IL: IIT Research Institute.

Levine, BS; Furedi, EM; Gordon, DE; Barkley, JJ; Lish, PM. (1990). Toxic interactions of the munitions compounds TNT and RDX in F344 rats. *Fundam Appl Toxicol* 15: 373-380.

Lish, P.M., B.S. Levine, E.M. Furedi, J.M. Sagartz and V.S. Rac (1984) Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse (Volumes 1 and 3). U.S. Army Medical Research and Development Command, Chicago, IL.

Ma, B. and H. Li (1993) Neurobehavior effects of hexogen on exposed workers. *Gongye Weisheng yu Zhiyebin* **19,** 20-23.

Major, MA; Reddy, G; Berge, MA; Patzer, SS; Li, AC; Gohdes, M. (2007). Metabolites profiling of [14C] hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Yucatan miniature pigs. *J Toxicol Environ Health A* 70: 1191-1202. http://dx.doi.org/10.1080/15287390701252717

Martin, D; Hart, E. (1974). Subacute toxicity of RDX and TNT in monkeys (pp. 1-216). (ADA044650). Kensington, MD: Litton Bionetics, Inc.

Marty, S; Wehrle, R; Sotelo, C. (2000). Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. *The Journal of Neuroscience* 20:8087-8095.

McCormick, NG; Cornell, JH; Kaplan, AM. (1981). Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Appl Environ Microbiol* **42,** 817-823.

Melnick, RL; Thaye, KA; Buche, JR. (2008). Conflicting views on chemical carcinogenesis arising from the design and evaluation of rodent carcinogenicity studies. *Environ Health Perspect* **116,** 130-135.

Merrill, SL. (1968). Ingestion of an explosive material, compostion C-4: A report of two cases. *USARV Med Bull* 40: 5-11.

Meyer, SA; Marchand AJ; Hight JL; Roberts GH; Escalon LB; Inouye LS; MacMillan DK. (2005). Up-and-down procedure (UDP) determinations of acute oral toxicity of nitroso degradation products of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Journal of Applied Toxicology* 25:427-434.

Mukhi, S; Patino,R. (2008). Effects of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in zebrafish: General and reproductive toxicity. *Chemosphere* 72:726-732.

Neal,GE; Judah, DJ; Hard, GG; Ito, N. (2003). Differences in ethoxyquin nephrotoxicity between male and female F344 rats. *Food Chem Toxicol* 41:193-200.

Neal, AP; Guilarte, TR. (2013). Mechanism of lead and manganese neurotoxicity. *Toxicol Res* 2:99-114.

Pan, X; M.J. San Francisco, MI; Lee, C; Ochoa, KM; Xu, X; Liu, J; Zhang, B; Cox, SB; Cobb, GP. (2007a) Examination of the mutagenicity of RDX and its N-nitroso metabolites using the Salmonella reverse mutation assay. *Mutat Res* **629,** 64-69.

Pan, X; Zhang, B; Smith, JN; San Francisco, M; Anderson, TA; Cobb, GP. (2007b). N-Nitroso compounds produced in deer mouse (Peromyscus maniculatus) GI tracts following hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) exposure. *Chemosphere* 67: 1164-1170. http://dx.doi.org/10.1016/j.chemosphere.2006.10.077

Pan X; Ochoa KM; San Francisco MJ; Cox SB; Dixon K; Anderson TA; Cobb GP. (2013). Absorption, distribution, and biotransformation of hexahydro-1,3,5-trinitro-1,3,5-triazine in B6C3F1 mice (Mus musculus). *Environmental Toxicology and Chemistry* 32:1295-1303.

Parker, GA; Reddy, G; Major, MA. (2006). Reevaluation of a twenty-four-month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse. *Int J Toxicol* **25,** 373-378.

Reddy, G; Erexson, GL; Cifone, MA; Major, MA; Leach, GL. (2005) Genotoxicity assessment of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Int J Toxicol* **24,** 427-434.

Rivera C; Voipio J; Payne JA; Ruusuvuori E; Lahtinen H; Lamsa K; Pirvola U; Saarma M; Kaila K. (1999). The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397:251-5.

Rogers JM, Chernoff N, Keen CL, Daston GP. (2005) Evaluation and interpretation of maternal toxicity in Segment II studies: Issues, some answers, and data needs. *Toxicology and Applied Pharmacology* 207:S367–74.

Schneider NR, Bradley SL, Andersen ME. 1978. Distribution and metabolism of cyclotrimethylenetrinitramine (RDX) in rat after sub-chronic administration. *Toxicol Appl Pharmacology* 46: 163-171.

Smith JN, Pan, XP; Gentles, A; Smith, EE; Cox, SB; Cobb, GE. (2006). Reproductive effects of hexahydro-1,3,5-trinitroso-1,3,5-triazine in deer mice (Peromyscus maniculatus) during a controlled exposure study. *Environ Toxicol Chem* 25: 446-451.

Smith JN; Espino MA, Liu J, Romero NA, Cox SB, Cobb GP. (2009). Multigenerational effects in deer mice (Peromyscus maniculatus) exposed to hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). *Chemosphere* 75: 910-914.

Suzuki, N; Withers HR. (1978). Exponential decrease during aging and random lifetime of mouse spermatogonial stem cells. *Science* 202: 1214-1215.

Suwa T, Nyska A, Peckham JC, Hailey JR, Mahler JF, Haseman JK, Maronpot RR.(2001) A retrospective analysis of background lesions and tissue accountability for male accessory sex organs in Fischer-344 rats. *Toxicol Pathol*. 29:467-78. PubMed PMID: 11560252.

Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling [Review] *Regul Toxicol Pharmacol* 62: 107-114. http://dx.doi.org/10.1016/j.yrtph.2011.12.007

Sweeney, LM; Okolica, MR; Gut, CP;, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). *Regul Toxicol Pharmacol* 64: 205-224.

Tan, EL; Ho, CH; Griest, WH; Tyndall, RL. (1992). Mutagenicity of trinitrotoluene and its metabolites formed during composting. *J Toxicol Environ Health A* 36: 165-175. http://dx.doi.org/10.1080/15287399209531632

Testud, F; Glanclaude, JM; Descotes, I. (1996). Acute hexogen poisoning after occupational exposure. *J Toxicol Clin Toxicol* 34: 109-111. http://dx.doi.org/10/3109/15563659609020244

U.S.EPA (1998). Health effects test guidelines: OPPTS 870.7800. Immunotoxicity. Washington, DC, United States Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances (EPA/712/C- 98/351; http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm)

U.S.EPA (2005). Guidelines for Carcinogen Risk Assessment. (EPA/630/P-03/0001F). Washington, DC U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S.EPA (2011). Recommended use of body weight$^{3/4}$ as the default method in derivation of the oral reference dose. (EPA/100/R11/0001). Washington, DC. U.S. Environmental Protection Agency, Risk Assessment Forum.

U.S.EPA (2012a). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC. U.S. Environmental Protection Agency. Risk Assessment Forum.

U.S. EPA (2012b). Peer review summary report. External letter peer review of study reports on 1,3,5-trinitro-1,3,5-hexahydrotriazine (RDX). Washington, DC. U.S. Environmental Protection Agency.

Von Oettingen, WF; Donahue, DD; Yagoda, H; Monaco, AR; Harris, MR. (1949). Toxicity and potential dangers of cyclotrimethylenetrinitramine (RDX). *J Ind Hyg Toxicol* 31: 21-31.

Wang, C; Leung, A; Sinhia-Hikim, AP. (1993). Reproductive aging in the male Brown-Norway rat: A model for the human. *Endocrinology* 133: 2773-2781.

Ward, JM; Goodman, DG; Squire, RA; Chu, KC; Linhart, MS (1978). Neoplastic and nonneoplastic lesions in aging (C57BL/6N × C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* 83; 849-854,

West, RR.; Stafford, DA. (1997) Occupational exposures and haematological abnormalities among ordnance factory workers: A case control study. *Leuk Res* 21**,** 675-680.

WHO (World Health Organization). (2012). Guidance for immunotoxicity risk assessment for chemicals. (Harmonization Project Document No. 10). Geneva, Switzerland. http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf

Williams, LR; Bannon, DI. (2009). Mechanism of RDX-induced seizures in rats (Toxicology Study No. 87-XE-OBT9-09). (ADA515072. USACHPPM-TSN-87-XE-OBT9-09). Aberdeen Proving Ground, MD: US Army Center for Health Promotion and Preventive Medicine, Health Effects Research Program. http://oai.dtic.mil/oai/oai?verb=getRecords&metadataPrefix=html&identifier=ADA511072

Williams, LR; Wong, K; Stewart, A; Suciu C; Gaikwad, S; Wu, N; DiLeo, J; Grossman, L; Cachat, J; Hart, P; Kalueff, AV. (2012). Behavioral and physiological effects of RDX on adult zebrafish. *Comparative Biochemistry and Physiology C-Toxicology and Pharmacology* 155:33-38.

Williams, LR; Aroniadou-Anderjaska, V; Qashu, F; Finne, H; Pidoplichko, V; Bannon, DI; Braga, MF.(2011). RDX binds to the GABA(A) receptor-convulsant site and blocks GABA(A) receptor-mediated currents in the amygdala: a mechanism for RD**X**-induced seizures. *Environ Health Perspect*. 119(3):357-63.

Wirbisky, SE; Weber, GJ; Lee, JW; Cannon, JR; Freeman, JL. (2014) Novel dose-dependent alterations in excitatory GABA during embryonic development associated with lead (Pb) neurotoxicity. *Toxicol Letters* 229(1):1-8.

Woody, RC; Kearns, GL; Brewster, MA; Turley, CP; Sharp, GB; Lake, RS. (1986). The neurotoxicity of cyclotriethylenetrinitramine (RDX) in a child: A clinical and pharmacokinetic evaluation. *Clin. Toxicol.* **24,** 305-319.

Zhang, X; Ebata, KT; Robaire, B; Nagano, MC. (2006). Aging of male germ line stem cells in mice. *Biol Reprod* 74: 119–124.

APPENDIX A: EPA'S CHARGE QUESTIONS

Draft Charge to the Science Advisory Board for the
IRIS Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) September 2016 (Updated November 2016[1])

Introduction

The U.S. Environmental Protection Agency (EPA) is seeking a scientific peer review of a draft Toxicological Review of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) developed in support of the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

IRIS is a human health assessment program that evaluates scientific information on effects that may result from exposure to specific chemicals in the environment. Through IRIS, EPA provides high quality science-based human health assessments to support the Agency's regulatory activities and decisions to protect public health. IRIS assessments contain information for chemicals that can be used to support hazard identification and dose-response assessment, two of the four steps in the human health risk assessment process. When supported by available data, IRIS provides health effects information and toxicity values for health effects (including cancer and effects other than cancer) resulting from chronic exposure. IRIS toxicity values may be combined with exposure information to characterize public health risks of chemicals; this risk characterization information can then be used to support risk management decisions.

An existing assessment for RDX includes a reference dose (RfD) posted on the IRIS database in 1988 and oral slope factor (OSF) and a cancer descriptor posted in 1990. The IRIS Program is conducting a reassessment of RDX. The draft Toxicological Review of RDX is based on a comprehensive review of the available scientific literature on the noncancer and cancer health effects in humans and experimental animals exposed to RDX. Additionally, appendices for chemical and physical properties, toxicokinetic information, summaries of toxicity studies, and other supporting materials are provided as *Supplemental Information* (see Appendices A to D) to the draft Toxicological Review.

The draft assessment was developed according to guidelines and technical reports published by EPA (see *Preamble*), and contains both qualitative and quantitative characterizations of the human health hazards for RDX, including a cancer descriptor of the chemical's human carcinogenic potential, a noncancer toxicity value for chronic oral exposure (RfD), and a cancer risk estimate for oral exposure.

Charge questions on the draft Toxicological Review of RDX

1. **Literature search/study selection and evaluation.** The section on *Literature Search Strategy | Study Selection and Evaluation* describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations including exclusion criteria, and study evaluation considerations, are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

2. **Toxicokinetic modeling.** In Appendix C, Section C.1.5, the draft assessment presents a summary, evaluation, and further development of published PBPK models for RDX in rats, mice, and humans (Sweeney et al., 2012a; Sweeney et al., 2012b).

2a. Are the conclusions reached based on EPA's evaluation of the models scientifically supported? Do the revised PBPK models adequately represent RDX toxicokinetics? Are the model assumptions and parameters clearly presented and scientifically supported? Are the uncertainties in the model appropriately considered and discussed?

2b. The average concentration of RDX in arterial blood (expressed as area under the curve) was selected over peak concentration as the dose metric for interspecies extrapolation for oral points of departure (PODs) derived from rat data. Is the choice of dose metric for each hazard sufficiently explained and appropriate? The mouse PBPK model was not used to derive PODs for noncancer or cancer endpoints because of uncertainties in the model and because of uncertainties associated with selection of a dose metric for cancer endpoints. Is this decision scientifically supported?

2c. In Section 2.1.3 of the draft assessment, an uncertainty factor of 10 for human variation is applied in the derivation of the RfD. Does the toxicokinetic modeling support the use of a different factor instead?

3. **Hazard identification and dose–response assessment.** In Chapter 1, the draft assessment evaluates the available human, animal, and mechanistic studies to identify health outcomes that may result from exposure to RDX. In Chapter 2, the draft assessment develops organ/system-specific reference values for the health outcomes identified in Chapter 1, then selects overall reference values for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/basic-information-about-integrated-risk-information-system#guidance) to reach the following conclusions.

   [Note: As suggested by the Chemical Assessment Advisory Committee panel that reviewed the draft IRIS assessment of benzo[a]pyrene, the charge questions in this section are organized by health outcome, with a question on each hazard identification followed by questions on the corresponding organ/system-specific toxicity values. This suggestion, however, entails some redundancy, as some questions apply equally to multiple health outcomes.]

3a. Nervous system effects

i. **Nervous system hazard** (Sections 1.2.1, 1.3.1)**.** The draft assessment concludes that nervous system toxicity is a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to the nervous system adequately assessed? Is there an appropriate endpoint to address the spectrum of effects?

ii. **Nervous system-specific toxicity values** (Section 2.1.1). Please comment on whether the selection of studies reporting nervous system effects is scientifically supported and clearly described. Considering the difference in toxicokinetics between gavage and dietary administration (described in Appendix C, Section C.1, and in the context of specific hazards in the toxicological review), is it appropriate to consider the Crouse et al. (2006) study, which used gavage administration? Is the characterization of convulsions as a severe endpoint, and the potential relationship to mortality, appropriately described?

iii. **Points of departure for nervous system endpoints** (Section 2.1.2). Is the selection of convulsions as the endpoint to represent this hazard scientifically supported and clearly described? Are the calculations of PODs for these studies scientifically supported and clearly described**?** Is the calculation of the HEDs for these studies scientifically supported and clearly described? Does the severity of convulsions warrant the use of a benchmark response level of 1% extra risk? Is calculation of the lower bound on the benchmark dose (BMDL) for convulsions appropriate and consistent with the EPA's Benchmark Dose Guidance?

iv. **Uncertainty factors for nervous system endpoints** (Section 2.1.3). Is the application of uncertainty factors to these PODs scientifically supported and clearly described? The subchronic and database uncertainty factors incorporate multiple considerations; please comment specifically on the scientific rationale for the application of a subchronic uncertainty factor of 1 and a database uncertainty factor of 3.[2]

v. **Nervous system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for nervous system effects scientifically supported and clearly characterized?

[2] Note that the database uncertainty factor applies to each of the hazards identified in the toxicological review.

3b. Kidney and other urogenital system effects

(i) **Kidney and other urogenital system hazard** (Sections 1.2.2, 1.3.1). The draft assessment concludes that kidney and other urogenital system toxicity is a potential human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this conclusion. Are all hazards to kidney and urogenital system adequately assessed? Is the selection of suppurative prostatitis as the endpoint to represent this hazard scientifically supported and clearly described?

(ii) **Kidney and other urogenital system-specific toxicity values** (Section 2.1.1). Is the selection of the Levine et al. (1983) study that describes kidney and other urogenital system effects scientifically supported and clearly described?

(iii) **Points of departure for kidney and other urogenital system endpoints** (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described**?** Is the calculation of the HED for this study scientifically supported and clearly described?

(iv) **Uncertainty factors for kidney and other urogenital system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

(v) **Kidney and other urogenital system-specific reference dose** (Section 2.1.4). Is the organ/system-specific reference dose derived for kidney and other urogenital system effects scientifically supported and clearly characterized?

3c. Developmental and reproductive system effects

(i) **Developmental and reproductive system hazard** (Sections 1.2.3, 1.3.1). The draft assessment concludes that there is suggestive evidence of male reproductive effects associated with RDX exposure, based on evidence of testicular degeneration in male mice. The draft assessment did not draw any conclusions as to whether developmental effects are a human hazard of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support these decisions. Are other hazards to human reproductive and developmental outcome adequately addressed?

(ii) **Reproductive system-specific toxicity values** (Section 2.1.1). Is the selection of the Lish et al. (1984) study that describes male reproductive system effects scientifically supported and clearly described?

(iii) **Points of departure for reproductive system endpoints** (Section 2.1.2). Is the calculation of a POD for this study scientifically supported and clearly described? Is the calculation of the HED for this study scientifically supported and clearly described?

(iv) **Uncertainty factors for reproductive system endpoints** (Section 2.1.3). Is the application of uncertainty factors to the POD scientifically supported and clearly described?

(v) **Reproductive system-specific reference dose** (Section 2.1.4). Is the organ/system- specific reference dose derived for reproductive system effects scientifically supported and clearly characterized?

3d. **Other noncancer hazards** (Sections 1.2.4, 1.2.6, 1.3.1). The draft assessment did not draw any conclusions as to whether liver, ocular, musculoskeletal, cardiovascular, immune, or gastrointestinal effects are human hazards of RDX exposure. Please comment on whether the available human, animal, and mechanistic studies support this decision. Are other non-cancer hazard adequately described?

3e. **Cancer**

(i) **Cancer hazard** (Sections 1.2.5, 1.3.2). There are plausible scientific arguments for more than one hazard descriptor as discussed in Section 1.3.2. The draft assessment concludes that there is *suggestive evidence of carcinogenic potential* for RDX, and that this descriptor applies to all routes of human exposure. Please comment on whether the available human, animal, and mechanistic studies support these conclusions.

(ii) **Cancer-specific toxicity values** (Section 2.3.1). As noted in EPA's 2005 *Guidelines for Carcinogen Risk Assessment*, "When there is suggestive evidence, the Agency generally would not attempt a dose-response assessment, as the nature of the data generally would not support one; however, when the evidence includes a well-conducted study, quantitative analyses may be useful for some purposes, for example, providing a sense of the magnitude and uncertainty of potential risks, ranking potential hazards, or setting research priorities." Does the draft assessment adequately explain the rationale for quantitative analysis, considering the uncertainty in the data and the suggestive nature of the weight of evidence, and is the selection of the Lish et al. (1984) study for this purpose scientifically supported and clearly described?

(iii) **Points of departure for cancer endpoints** (Section 2.3.2, 2.3.3). Are the calculations of PODs and oral slope factors scientifically supported and clearly described?

4.**Dose-response analysis.** In Chapter 2, the draft assessment uses the available human, animal, and mechanistic studies to derive candidate toxicity values for each hazard that is credibly associated with RDX exposure in Chapter 1, identify an organ/system-specific RfD, then selects an overall toxicity value for each route of exposure. The draft assessment uses EPA's guidance documents (see http://www.epa.gov/iris/basic-information-about-integrated-risk- information-system#guidance) in the following analyses.

4a. **Oral reference dose for effects other than cancer** (Sections 2.1.5–2.1.8). The draft assessment presents an overall oral reference dose of 3×10^{-3} mg/kg-day, based on nervous system effects as described in the Crouse et al. (2006) study. Is this selection scientifically supported and clearly described, including consideration of mortality as described in Section 2.1.6, and consideration of the organ/system-specific reference dose derived from the toxicity study by Cholakis et al. (1980) that is lower (by approximately fivefold) as described in Section 2.1.4?

4b. **Inhalation reference concentration for effects other than cancer** (Section 2.2). The draft assessment does not derive an inhalation reference concentration as the available studies were insufficient to characterize inhalation hazard and conduct dose-response analysis, and no toxicokinetic studies of RDX were available to support development of a PBPK inhalation model. If you believe that the available data might support an inhalation reference concentration, please describe how one might be derived.

4c. **Oral slope factor for cancer** (Section 2.3.3–2.3.4). The draft assessment presents an overall oral slope factor of 0.038 per mg/kg-day based on the combination of liver and lung tumors in female mice. Is this derivation scientifically supported and clearly described?

4d. **Inhalation unit risk for cancer** (Section 2.4). The draft assessment does not derive an inhalation unit risk because inhalation carcinogenicity data were not available, nor were toxicokinetic studies of inhalation of RDX available to support development of an inhalation PBPK model. If you believe that the available data might support an inhalation unit risk, please describe how one might be derived.

5.**Executive Summary**. Does the executive summary clearly and adequately present the major conclusions of the assessment?

References

Cholakis, JM; Wong, LCK; Van Goethem, DL; Minor, J; Short, R; Sprinz, H; Ellis, HV, III. (1980). Mammalian toxicological evaluation of RDX. (DAMD17-78-C-8027). Kansas City, MO: Midwest Research Institute.

Crouse, LCB; Michie, MW; Major, M; Johnson, MS; Lee, RB; Paulus, HI. (2006). Subchronic oral toxicity of RDX in rats. (Toxicology Study No. 85-XC-5131-03). Aberdeen Proving Ground, MD: U.S. Army Center for Health Promotion and Preventive Medicine.

Levine, BS; Lish, PM; Furedi, EM; Rac, VS; Sagartz, JM. (1983). Determination of the chronic mammalian toxicological effects of RDX (twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the Fischer 344 rat): Final report--phase V. Chicago, IL: IIT Research Institute.

Lish, PM; Levine, BS; Furedi, EM; Sagartz, JM; Rac, VS. (1984). Determination of the chronic mammalian toxicological effects of RDX: Twenty-four month chronic toxicity/carcinogenicity study of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in the B6C3F1 hybrid mouse (Volumes1-3). (ADA181766. DAMD17-79-C-9161). Fort Detrick, Frederick, MD: U.S. Army Medical Research and Development Command. http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA181766

Sweeney, LM; Gut, CP, Jr; Gargas, ML; Reddy, G; Williams, LR; Johnson, MS. (2012a). Assessing the non-cancer risk for RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) using physiologically based pharmacokinetic (PBPK) modeling [Review]. Regul Toxicol Pharmacol 62: 107-114. http://dx.doi.org/10.1016/j.yrtph.2011.12.007

Sweeney, LM; Okolica, MR; Gut, CP, Jr; Gargas, ML. (2012b). Cancer mode of action, weight of evidence, and proposed cancer reference value for hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Regul Toxicol Pharmacol 64: 205-224. http://dx.doi.org/10.1016/j.yrtph.2012.07.005

APPENDIX B: EDITORIAL COMMENTS

Specific comments on text and presentation of data on reproductive and developmental toxicity

Page 1-39, line 30: Reference to historic controls should be deleted. It is not valid to compare with historical controls (Ward et al., 1979) because it was done by different investigators and no quantitative level of what constitutes testicular degeneration is presented.

Page 1-39, line 33: Add "and a 14% decrease in testis weight" It is useful to add this because this was the only study in which testis weight showed a corresponding decrease with germ cell degeneration, as would be expected.

Page 1-40, line 4: Delete text and references about increases in testis weights. The only significant increase was for relative testis weight and this was really due to a loss of body weight.

Page 1-40, lines 8-21: The presentation and discussion of the results of the two-generation and dominant lethal studies should be combined. They were not separate studies; in fact the same male rats were used for both. Moreover, they show that same result: decrease in yield of pregnancies from males exposed to 50 mg/kg-day. The only difference was the treatment of the females.

Specific comments on presentation of data (Tables, Figures) on reproductive and developmental toxicity

1. The Tables and Figures are well planned and show the important features that need to be presented. However we believe that Table 1 of this report should be added as it compares all the rodent studies in one table, facilitating comparisons showing support and discrepancy.

2. In Table 1-9, the relative testes weights should be deleted. Relative testis weight is affected by changes in body weights, which in our experience does not have effects on testis weights of adult animals. Absolute testis weights are a better measure of testicular toxicity of an agent. The relative testis weights just clutter up the table and add little information on the toxicity of RDX.

3. Table 1-9, Page 1-42: In the presentation of the data of Levine et al. (1983), the data on "SDMS" (spontaneous death or moribund sacrifice) rats should be deleted. Their significance is open to question and they aren't given much weight in the discussion.

4. Table 1-9, Page 1-44: The data on incidence of germ degeneration of Levine et al. (1981a,b, 1990) at 12 and 15 mg/kg-day should be deleted. These were observed on dead rats (all rats in these groups died). Incidentally the numbers were reversed: the value for 1/10 was for the 12 mg/kg-day dose and 1/9 was for 15 mg/kg-day.

5. Table 1-9 (footnote, Page 1-44) Also reference to historic controls for comparison of testicular degeneration reported by Lish et al. (1984) should be deleted.

6. The testis weight data from Cholakis et al. (1980) (Table 1-9, last entry on Page 1-43) on F2 weanlings does not belong in the male reproductive effect section. It is not indicative of direct effect on testis weight and there is no follow-up to determine whether or not adult testis weights will be affected. Rather it belongs in the developmental effects section (Table 1-10).

7. Figure 1-3 could provide a useful comparison of doses from various studies. However, to achieve maximum impact, the data should be grouped as follows: mouse; rat 2-year chronic; rat 13-week subchronic. The study using gavage should be noted since the effective dose seems to be dependent on method of oral administration. Footnote (1), indicating that the non-significant change in testis weight in Hart et al. (1976) was a slight increase, was confusing and should be deleted; anyway that is covered by the symbol that there was no significant change.

8. Figure 1-3. Additionally, it may be a matter of rote procedure, but the decision to highlight only statistically significant findings in the exposure-response array is deceptive because the two studies identified with statistical significant findings (Levine 1990 and 1983) were not considered meaningful results, but the nonstatistically significant finding in Lish et al. (1984) is the finding for male reproductive effects that is debated heavily in this document and is not highlighted. Perhaps add an explanation via footnotes.

9. Table 1-10 Page 1-46: Reconsider the use of term ‘offspring survival’ to categorize ‘prenatal mortality’ as offspring survival is more commonly associated with postnatal outcome.

10. Figure1-4: typo in spelling of ‘significantly’ in the key.

APPENDIX C: SUGGESTIONS ON THE FORMAT FOR EPA's CHARGE QUESTIONS

The CAAC-RDX panel has the following observations on the charge questions based on experience during the review meeting:

1) Charge questions on the calculation of points of departure for organ/system-specific reference dose did not account for the possibility that the panel may not agree with the selection of the specific endpoint for derivation of a POD (as is the case for the use of suppuratitive prostatitis for derivation of a POD for kidney and other urogenital system effects, and the use of testicular degeneration for the derivation of a POD for male reproductive effect).

Suggestions

- There should be a question if the panel agrees with the selection of a specific endpoint for derivation of a POD, before the question if the calculation of the POD is scientifically supported and clearly described.
- There should also be a question on whether there is an alternative approach.